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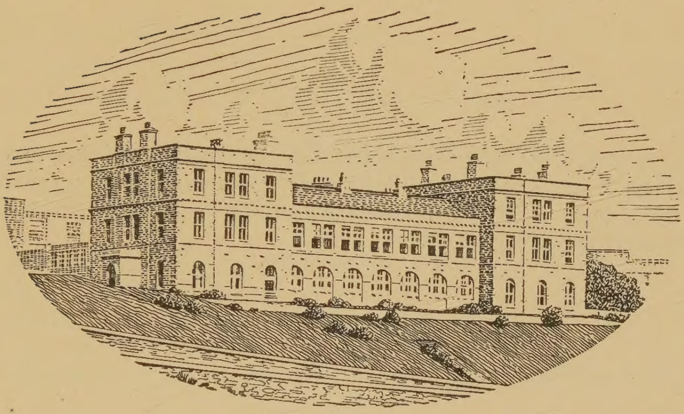

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Page 589. Title, line 2. *For* XVI *read* XV.
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320. Section heading. For *Cercariae* read *Cercaria.*
592. Bottom line. For *Phycodris* read *Phycodrys.*
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THE PROBOSCIS APPARATUS OF *ARENICOLA*

By G. P. Wells

Department of Zoology, University College, London

(Plates I–IV and Text-figs. 1–14)

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INTRODUCTION

The anterior end of *Arenicola* contains an elaborately organized apparatus which has hitherto escaped accurate description, although it is of evident importance in the processes of extrusion and withdrawal of the proboscis, and exhibits striking variations within the genus as at present constituted. The 'proboscis apparatus', as it will be termed, includes the following components: (i) the proboscis itself and the first part of the oesophagus, together constituting the anterior portion of the gut; and (ii) the retractor sheath and gular membrane ('first septum' or 'first diaphragm' of previous authors), both of which, in the writer's view, are derived from the first septum. Although of diverse morphological origin, these components are built together into a functionally unified whole. The purpose of the following paper is to give an account of the anatomy of the apparatus on which later studies of its mode of action can be based. The three species to be described, *A. ecaudata* Johnston, *A. claparedii* Levinsen and *A. marina* L., are chosen to represent the three sections into which the genus naturally falls. It will be shown that the proboscis apparatus undergoes characteristic structural modifications in each of the three, although its basal plan is always the same. So great are the divergences that probably each of them extrudes its proboscis in a different way.

A revision of the external characters, body wall and parapodia of the same three species was recently published (Wells, 1950). The material and anatomical methods described in that paper apply also to the present one, except that I have received some excellent additional material of *A. claparedii* from Departure Bay, Nanaimo, by the kindness of Dr Peter Ford, of the Department of Zoology, University of British Columbia.

The chief point of difficulty in studying the anatomy of the proboscis apparatus is the diversity of positions which it can assume. The proboscis may be wholly or partly extruded, or withdrawn. When withdrawn its arrangement, and that of the associated structures, can vary greatly according to the configuration of the anterior end as a whole. Most of the anatomical descriptions refer to worms killed in one or other of two attitudes, termed 'relaxed' and 'distended'. Details of the methods of killing are given in the earlier paper. A relaxed worm is in a tolerably normal attitude, with the proboscis partly or wholly withdrawn. A distended worm has been narcotized and its body cavity blown up with fixative at the moment of killing. Its proboscis is extruded and all the parts are stretched to an abnormal degree, but the result is useful for the elucidation of many of the finer anatomical details. Except where otherwise stated, the descriptions refer to relaxed worms.

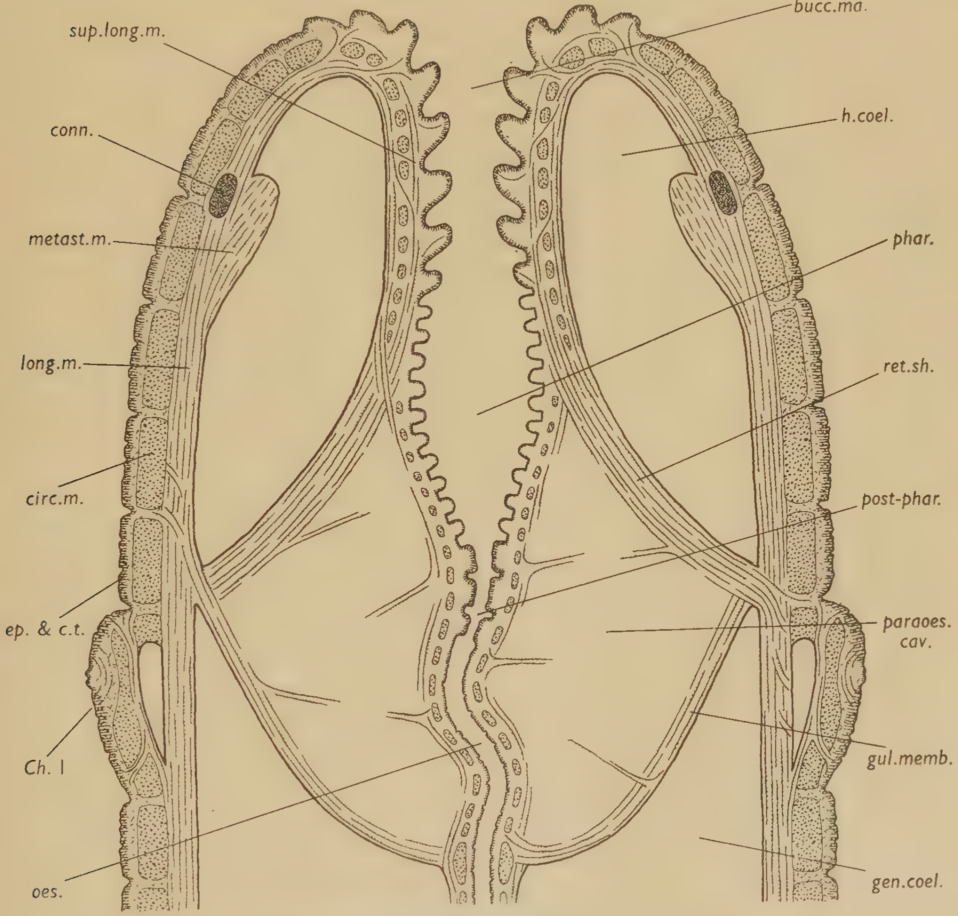
General Account of the Proboscis Apparatus

The three species to be considered represent divergent specializations of a common pattern. An account will first be given of the general plan of the proboscis apparatus, and afterwards the modifications shown by the three species will be described. The general account applies to all of the species, except where otherwise stated.

The simplest way to see the plan of the proboscis apparatus is to bisect the anterior end horizontally with a razor blade and examine it with a dissecting microscope (Pl. II, fig. 3). Sagittal sections are often confusing because of certain elaborations which appear in the median plane. A generalized horizontal section, with the proboscis withdrawn, is shown in Text-fig. 1, and described in the following paragraphs.

The 'head' of the worm is the bluntly conical region extending forwards from the anterior margin of the first chaetigerous annulus (*Ch.* I). Its body wall consists of the following layers: (i) epidermis (*ep.*); (ii) sub-epithelial connective tissue (*c.t.*); (iii) circular muscle (*circ.m.*); (iv) a layer of intermuscular connective tissue; (v) longitudinal muscle (*long.m.*); (vi) coelomic epithelium. The ventral nerve cord passes forward on to the head and divides into two connectives (*conn.*) which run upwards and forwards to the brain. The position of the nerve cord and connectives relative to the layers of the body wall varies from species to species. The connectives are accompanied by the metastomial muscles (*metast.m.*), which are derived from the longitudinal layer and pass obliquely upwards to meet each other immediately ventral to the brain. The structure of the body wall of the head was described in fuller detail elsewhere (Wells, 1950).

The proboscis is the eversible part of the gut, and consists of: (i) a buccal mass, with large, prominent papillae whose form varies greatly from species to species (*bucc.ma.*); (ii) a pharynx, with finer, more closely set papillae (*phar.*); (iii) a short post-pharynx with rather larger papillae (*post-phar.*). The post-pharynx is the extreme limit of that part of the gut which is turned inside-out during extrusion. It is followed by the more or less laterally compressed oesophagus (*oes.*).


sup.long.m.
conn.
metast.m.
long.m.
circ.m.
ep. & c.t.
Ch. I
oes.
bucc.ma.
h.coel.
phar.
ret.sh.
post-phar.
paraoes.
cav.
gul.memb.
gen.coel.

Text-fig. 1. Generalized horizontal section through the anterior end of *Arenicola*. Lettering as on p. 28.

The body wall continues into the proboscis at the mouth, and the walls of the proboscis and oesophagus are composed of layers having fundamentally the same arrangement as those of the body wall. There are, however, local specializations, of which the most striking is the presence in the buccal mass and the oral end of the pharynx of a superficial longitudinal muscle layer, lying between the circular muscle and the epithelium (*sup.long.m.*).

The individual proboscis papillae appear at first sight to be hollow outpushings of the gut wall. They are, however, more correctly described as thickenings of the wall which have become hollowed by the great development of the interstitial spaces between the cells (Pl. IV, figs. 1, 2). They are bounded, on the surface towards the gut, by the epithelium and a thin layer of underlying connective tissue; they contain blood vessels and muscle fibres, the latter partly derived from the main muscle layers of the gut and partly intrinsic to the individual papillae. Because of these muscle fibres, and the extensive interstitial spaces, they have considerable powers of movement. Ideally, the spaces are cut off from the true coelom by the lining of the latter cavity, but the actual relationships vary from species to species, and from region to region of the proboscis.

The pharyngeal papillae vary somewhat in size, small ones being scattered about among their larger neighbours. This fact suggests that new papillae are intercalated all over the surface of the pharynx as the worm grows. The buccal papillae are of very uniform size, except that those at the oral and pharyngeal ends of the buccal mass are rather smaller than those in the middle. The growth of the buccal mass seems therefore to occur by increase in size of the individual papillae, and not by the intercalation of new ones among the old. The buccal mass and pharynx are separated by a more or less extensive transition zone bearing papillae of the pharyngeal type intermixed with others of the buccal type; the latter decrease in size from the buccal to the pharyngeal end of the transition zone and are finally no larger than the pharyngeal papillae, into which they merge (Pl. I, fig. 1). The appearances could be due to a continual conversion of pharynx into buccal mass, taking place at the boundary between them.

The gut is suspended from the body wall of the head by two transverse sheets of tissue, the retractor sheath (*ret.sh.*) and the gular membrane (*gul.memb.*). The latter is the 'first diaphragm' or 'first septum' of Ashworth (1904, 1912), but for reasons explained below I have substituted a name carrying no implication of serial homology.

The retractor sheath consists of coelomic epithelium, connective tissue and a single muscle layer in that order, the muscle layer lying on its hinder face. My sections—the thinnest of which are 15μ, as they were made for fine anatomy rather than histology—also show a delicate endothelial layer on the hind face of the muscle in *ecaudata*, but not with any certainty in *claparedii* or *marina*. The muscle fibres run radially from the body wall to the gut, which they reach about half-way along the pharynx. Most of them then pass through the circular muscle layer of the gut to constitute the superficial longitudinal layer of the proboscis; the details of this region are, however, complicated and will be returned to in the detailed descriptions of the three species.

The gular membrane consists of the same layers as the retractor sheath, but in the reverse order—i.e. with the muscle layer in front of the connective tissue and epithelium. It varies greatly from species to species, both in general form and in the massiveness of its musculature. The latter runs radially in *claparedii* and *marina*, but is elaborated into a radial layer lying anterior to a circular one in *ecaudata*.

The retractor sheath and gular membrane are inserted together on the body wall, just in front of the first chaetigerous annulus. Their relations at this point can be imitated by tightly clasping one's hands and then extending the fingers. The palms of the hands represent the two sheets, and the fingers are the columns into which their radial muscle layers continue peripherally. The columns decussate and immediately enter the body wall. At first sight they appear to join the longitudinal layer, but serial sections show that a large part, at least, of their fibres make their way through to the connective tissue around and between the circular muscle bundles.

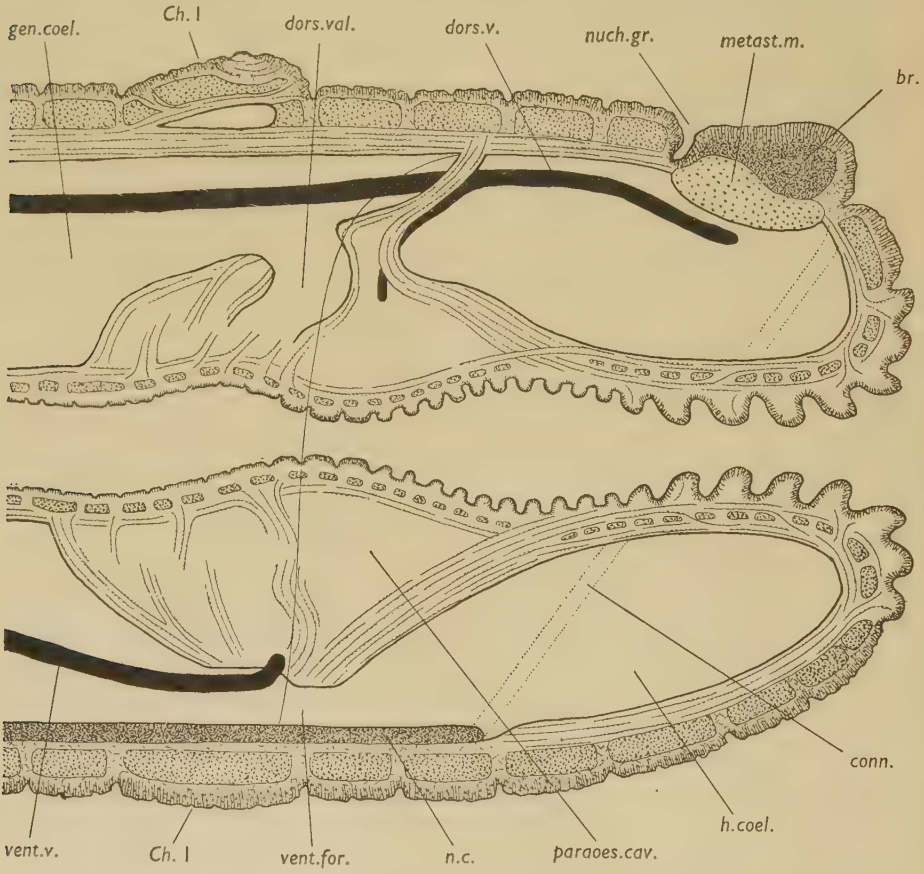
The horizontal section shows three large cavities between the gut and the body wall. These are the general coelome behind the gular membrane (*gen.coel.*), the head coelome in front of the retractor sheath (*h.coel.*), and the paraoesophageal cavity between the two sheets (*paraoes.cav.*). The paraoesophageal cavity contains a loose network of blood vessels lying freely within it, and is crossed by numerous very fine strands of muscle, the radial strands, which detach themselves from the retractor sheath and gular membrane and run to the wall of the gut.

The gular membrane and retractor sheath are imperforate, and therefore isolate the paraoesophageal cavity completely from the other cavities, except at certain special points as described below. The head coelome is separated from the general coelome by the peripheral muscle columns of the two sheets as they cross from their decussation to the body wall—the bases of the fingers, in the analogy of the clasped hands—but this barrier is never complete. Fluid can flow between head coelome and general coelome to an extent which varies, not only according to specific differences in form, but also according to the degree of contraction of the muscle columns.

On examining sagittal sections, or a sagittally dissected worm, certain additional points can be seen. A generalized sagittal section is shown in Text-fig. 2. The position of the central nervous system is given by the ventral nerve cord (*n.c.*), the brain (*br.*), and the dotted lines indicating the course of the connectives (*conn.*).

The position of the insertion of the retractor sheath and gular membrane on the body wall is shown as a fine ruled line. Ventrally and laterally, it is placed at or near the front border of the first chaetigerous annulus, but as the mid-dorsal line is approached it curves forwards to form, with its fellow of the opposite side, an anteriorly directed V, the apex lying about half-way between the first chaetigerous annulus (*Ch.* I) and the nuchal groove (*nuch.gr.*). The line varies slightly in position from species to species.

The insertion of the retractor sheath and gular membrane is interrupted ventrally, in such a way as to leave a ventral foramen (*vent.for.*) through which the general coelome and head coelome communicate. This foramen is bounded laterally by the insertions of the two sheets, and could presumably be closed by contraction of their muscle columns. Its roof is the floor of the paraoesophageal cavity, which is thin and membranous in the mid-ventral line, the muscle columns having diverged to reach the body wall lateral to the foramen.



Text-fig. 2. Generalized median sagittal section through the anterior end of *Arenicola*. Lettering as on p. 28.

The ventral vessel (*vent.v.*) ends anteriorly by passing upwards through the floor of the paraoesophageal cavity in the region of the ventral foramen, and breaking up into branches which join the vascular network lying in that cavity.

The radial muscle strands which cross the paraoesophageal cavity are especially well developed in the median plane. In particular, one or more thickened strands run from the lateral wall of the ventral foramen to the gut, which they reach just behind the post-pharynx. These strands may be regarded as a ventral accessory retractor of the proboscis.

Mid-dorsally, the gular membrane is thrown into a vertically descending fold, the dorsal valve (*dors.val.*). This fold is narrow from side to side, so that it has the general form, in horizontal section, of a flattened tube. The muscular layer of its lateral walls passes down as a series of strands to reach the gut just behind the post-pharynx; they probably act collectively as a dorsal accessory retractor of the proboscis. The membranous layer of the fold is continuous in its dorsal part but becomes perforated more ventrally, as it nears the gut. Body fluid can therefore make its way down the fold from the general coelome into the paraoesophageal cavity—a fact which explains the presence in the latter of coelomic corpuscles and developing germ cells identical with those found in the general coelome—but, owing to the lateral flattening of the tube, the passage of fluid in the reverse direction is presumably difficult.

The dorsal vessel (*dors.v.*) passes forwards through the insertion of the retractor sheath and gular membrane. As it does so, it gives off one or more branches which run downwards and backwards into the paraoesophageal cavity to join its vascular network.

SERIAL HOMOLOGY OF THE PROBOSCIS APPARATUS

Ashworth's well known monograph of *A. marina* contains the following passage (Ashworth, 1904, p. 19):

> 'The coelom is spacious and continuous from one end of the animal to the other. In front it is sub-divided transversely by three fenestrated septa, or diaphragms. The first of these is placed at the level of the anterior edge of the first chaetigerous annulus.... The second and third diaphragms mark the posterior limits of the second and third chaetigerous segments.'

The first of these diaphragms is our gular membrane. Ashworth evidently regarded all of the diaphragms as serially homologous with each other and as representing true septa (see also his pp. 22–4).

Ashworth's knowledge of the anatomical relationships inside the head was not very exact. In particular, he was unaware of the existence of the paraoesophageal cavity as a definitely walled-off space. He described the first diaphragm incorrectly as fenestrated, or 'perforated by numerous, rounded, usually oval apertures', as the third is, and he drew the retractor sheath, as it runs forwards from its decussation with the gular membrane, in the form of a series of separate slips instead of a continuous sheet (see his plate III, fig. 23). Moreover, he said nothing about the serial homology of the retractor sheath. As this structure meets the body wall just behind the gular membrane and the gut wall in front of the membrane, its derivation presents a difficult morphological problem if the gular membrane is in fact a septum.

Lillie (1905) published an account of the development of *A. cristata* Stimpson, a species which closely resembles *A. marina*. His main interest was in the nephridia, and he apparently relied for the rest of the adult anatomy on the works of Gamble & Ashworth (1898, 1900), on which Ashworth's monograph of 1904 is largely based. Lillie believed, contrary to Ashworth, that the first diaphragm has a different origin from the other two, and that none of them represent the primitive septa. His views on the second and third are irrelevant to the present discussion. With regard to the first diaphragm, or gular membrane, he wrote as follows (his p. 353):

> 'The most anterior septum occupies from the first a position immediately behind the line of insertion of the proboscideal retractor muscles (plate 24, fig. 35). As growth proceeds, the relations of these two originally independent structures become closer, and at the stage represented in fig. 36—in which twelve somites have been laid down—the two have become intimately associated with one another, though still distinguishable on close examination. This association of proboscideal muscles and first septum becomes in later stages more complete, and both eventually enter into the formation of the muscular first diaphragm. The posterior peritoneal wall of the adult diaphragm may be regarded as representing the original first septum.'

Lillie gave no information about the origin of the retractor sheath, whose first appearance in his account is as quoted above. Moreover, I have failed to find conclusive evidence for his interpretation in his published illustrations: this point is returned to below.

Ashworth afterwards accepted Lillie's views on the nature of the first diaphragm (Ashworth, 1912, pp. 61–2).

My own preference is for a third hypothesis, which may be stated as follows. The first chaetigerous segment was originally bounded anteriorly by a septum inserted on the body wall at the front border of the first chaetigerous annulus. This septum would consist of two layers of coelomic epithelium separated by connective tissue with muscle fibres and blood vessels in it. Let us assume that a cleft—the paraoesophageal cavity—has appeared in the middle layer of the septum, splitting it into two sheets. The anterior sheet becomes the retractor sheath, and the posterior sheet the gular membrane. If this be true, the paraoesophageal cavity is not coelomic.

This hypothesis has the advantage over its predecessors of finding a place for the retractor sheath in the segmental scheme. It explains why the sheath and the gular membrane are inserted at the same level on the body wall. The forward displacement of their plane of insertion in the mid-dorsal region supports the hypothesis, for a tendency to forward movement of the dorsal part of the septal insertions relative to the external annulation is visible in other segments of *Arenicola*. Moreover, the retractor sheath and gular membrane are so intimately related at certain points, notably in the median plane, that it is impossible to tell where one leaves off and the other begins. This might perhaps be due to the blending of two originally distinct structures, as Lillie suggested, but it is more simply explained if the sheath and membrane are in fact one structure.

The paraoesophageal cavity differs from the coelome in being crossed by the numerous radial muscle strands, and also in containing a network of vessels, lying freely in its cavity, to which both the dorsal and ventral vessels contribute. In other segments the dorsal and ventral vessels have no direct communication with each other except by means of commissural loops running in the septa,[1] and if the paraoesophageal cavity represents a cleft within a septum, its vascular network can be derived from the commissural loop. The presence or absence of an epithelial lining to the cavity cannot be taken as evidence, for on the one hand, the coelomic epithelium can break down and disappear locally (e.g. in the buccal region of *claparedii* and *marina*), while on the other, many non-coelomic cavities, such as blood vessels, may acquire endothelial linings. The following point is, however, suggestive. My sections of *claparedii* show conspicuous brown 'chlorogogenous' cells on the ventral vessel. These cells, which are presumably derived from the coelomic epithelium, continue forwards exactly to the point of entry of the ventral vessel into the paraoesophageal cavity, where they end abruptly.

Although the hypothesis is in good agreement with the adult anatomy, it is rather in the early development that conclusive proof of its truth should be sought. Here I have no direct experience; but I have tried without success to find decisive evidence in Lillie's figures in favour either of his interpretation or of mine. The passage quoted above refers to his figs. 35 and 36, which respectively show 8- and 12-somite larvae. Unfortunately, they are both sagittal sections and, because of the dorsal valve and the other complications which appear in the median plane, neither of them shows the relations of the retractor sheath and gular membrane at all clearly. If we turn to his figures of earlier stages, we find sections of 5- and 6-somite larvae in his figs. 7 and 8, plate 22. These are again sagittal, and their dorsal sides are difficult to understand, but their ventral sides are suggestive. In the 5-somite larva ('swarming stage') the mouth is still closed. The future proboscis appears to be connected to the body wall by a thin membranous septum only. In the 6-somite larva ('beginning of crawling stage') the mouth is open and the proboscis has either just become, or is about to become, eversible (see his p. 347). The septum has now thickened into a broad wedge with its apex on the body wall and its base on the gut. This looks like the separation of a single septum into two sheets, rather than the merging of two originally distinct structures.

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[1] These loops can be seen very clearly in the hinder branchiate segments of *ecaudata*, by isolating a single segment and examining in end view. The loops supply the gills and give off branches to the main longitudinal vessels of the body wall. The loops often persist even where the septa have disappeared, and are variously modified in the different regions of the body. That of the (vanished) septum vii forms the so-called ventricles. I hope to describe the loops in detail in a later work.

By the kindness of Dr Helen Pixell Goodrich, I have been allowed to use some horizontal sections of a 1·5 mm. *A. cristata* post-larva from Woods Hole, made by the late Prof. Goodrich. The tail is already nearly complete in this animal. A section of the anterior half, passing just ventral to the mouth, is shown in Pl. II, fig. 4. The metamerically arranged septa are clearly visible. If the convention is adopted that the septum at the anterior boundary of the *n*th chaetigerous segment is called the *n*th septum, then those marked *D.* II and *D.* III, which will become the 'second and third diaphragms' of the adult, are in reality the third and fourth septa. The second, marked *D.vest.*, will become a vestigial diaphragm which can be clearly seen in the adult, connecting the corresponding segmental vessels to the body wall. One segment farther forwards, the future retractor sheath and gular membrane meet the body wall, and the suggestion seems irresistible that they both represent the first septum. Conclusive proof could perhaps be found in horizontal sections of earlier stages, with 5 or 6 somites. There is at least a possibility that the first septum would be seen in the act of division in such material.

MODIFICATIONS OF THE PROBOSCIS APPARATUS IN *ARENICOLA ECAUDATA* JOHNSTON, *A. CLAPAREDII* LEVINSEN, AND *A. MARINA* L.

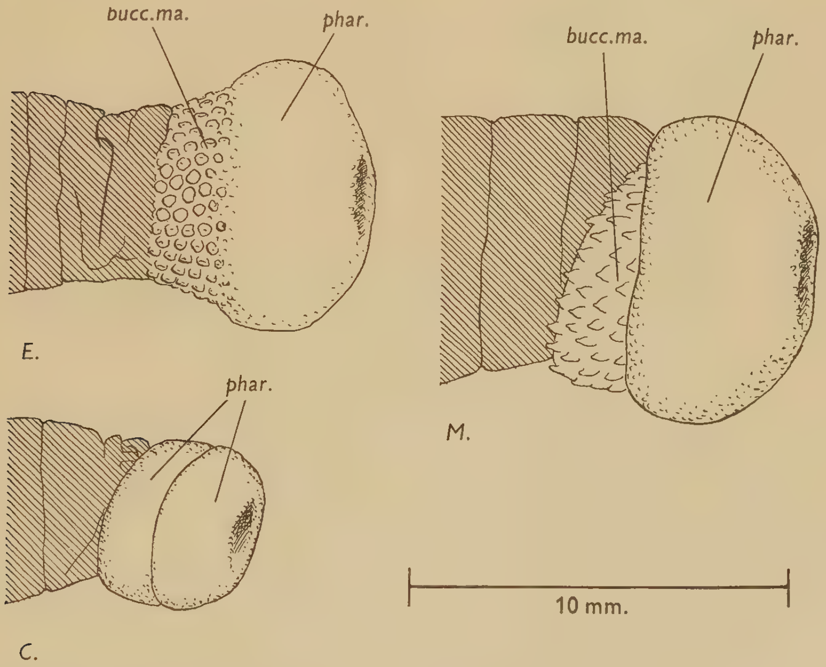
The description of the proboscis apparatus in the three species follows a comparative plan, those parts of the apparatus which show important modifications being taken in order.

The Proboscis

The proboscis varies from species to species in general form and also in the form of the papillae.

The extruded proboscis of *ecaudata* generally has the form of a very flat cone, rather like a loudspeaker cone, with a slightly domed top—the cone being the buccal mass and the top the pharynx. The animals drawn in Text-figs. 3*E.* and 12*E.* were artificially distended after narcosis and show an extreme degree of inflation which is seldom or never attained in the living animal. The pharyngeal papillae are conical. Those of the buccal mass appear, when the proboscis is fully distended, as rounded or polygonal raised areas with flat tops. In the partly distended or withdrawn organ the centres of the tops sink down so that the buccal papillae are sucker-shaped (Text-fig. 4 *E.*;

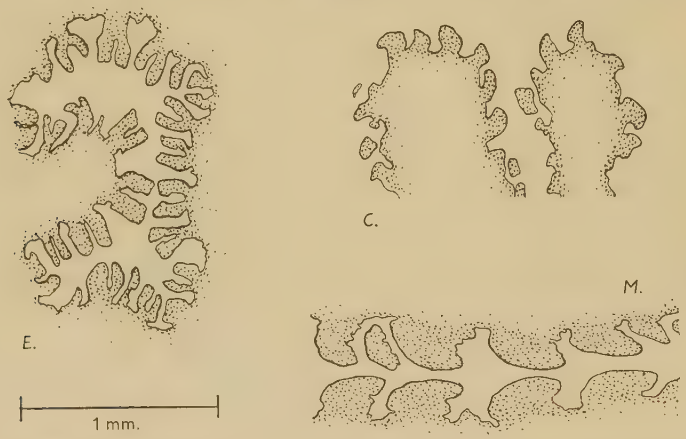
Pl. I, fig. 1; Pl. II, fig. 1). The arrangement of their musculature indicates that a sucker action is of real importance in their normal functioning, for there are strands running down from the tops to the deeper layers of the gut wall, which could cause depression, and others running across on the deep surfaces of the tops, which could cause raising and flattening (Pl. IV, fig. 1). The contrast between the sucker-like buccal papillae of *ecaudata* and the tooth-like ones of the other two species is very great. The transition zone between buccal mass and pharynx, in which papillae of the two types are intermingled, is broad in *ecaudata*.



Text-fig. 3. Lateral views of the anterior ends of distended specimens of *ecaudata* (*E.*), *claparedii* (Neapolitan specimen) (*C.*) and *marina* (*M.*). Other lettering as on p. 28.

The pharynx of *claparedii* is relatively more extensive than that of *ecaudata*, and is blown out, at the moment of full extrusion, into a rounded, bubble-like form, which overlaps and conceals the buccal mass. The drawing of Text-fig. 3*C.* was made from an artificially distended specimen, but the form of the pharynx resembles that normally seen in the living worm. The expanded pharynx is characterized by a 'waist-line' which corresponds to the attachment of the retractor sheath muscles on its inner surface. The pharyngeal papillae are more spherical than those of *ecaudata*. The buccal papillae are stout conical teeth, with their apices directed towards the mouth (Text-fig. 4 *C.*).[1] There is a broad transition zone between buccal mass and pharynx, as in *ecaudata* (Pl. I, fig. 2).

The proboscis of *marina* resembles that of *claparedii* in its pharnyx which assumes a rounded form at full extrusion, and in its conical buccal papillae directed towards the mouth (Pl. I, fig. 3; Pl. II, fig. 2; Pl. IV, fig. 2). Its pharynx differs from that of *claparedii* in lacking the 'waist-line' and also in being slightly less extensive, so that it overlaps the buccal mass less completely at the moment of full extrusion. On the other hand, the buccal papillae of *marina* are larger and more powerful-looking than those of *claparedii*. I have suggested elsewhere that the buccal mass of *marina* is used as a rasp in burrowing (Wells, 1948). The boundary between buccal mass and pharynx is relatively abrupt in *marina*; the transition zone exists, but is much narrower than in the other species.



Text-fig. 4. Camera lucida outlines of microtome sections, to show the form of the buccal papillae. *E.*, *ecaudata*: transverse section through withdrawn buccal mass. *C.*, *claparedii* (Neapolitan specimen): horizontal section through partly extruded buccal mass. *M.*, *marina*: sagittal section through withdrawn buccal mass, the oral end to the right.

The Buccal Peritoneum

As already noted, the proboscis papillae are hollowed out by the great development of their interstitial spaces. The relations between these spaces and the main body cavities are of evident functional interest. In the part of the gut which traverses the paraoesophageal cavity, the interstitial spaces open freely into that cavity; this is to be expected if it is itself an enormous interstitial cleft. The relations of the spaces in the buccal papillae to the head coelome vary from species to species, as follows.

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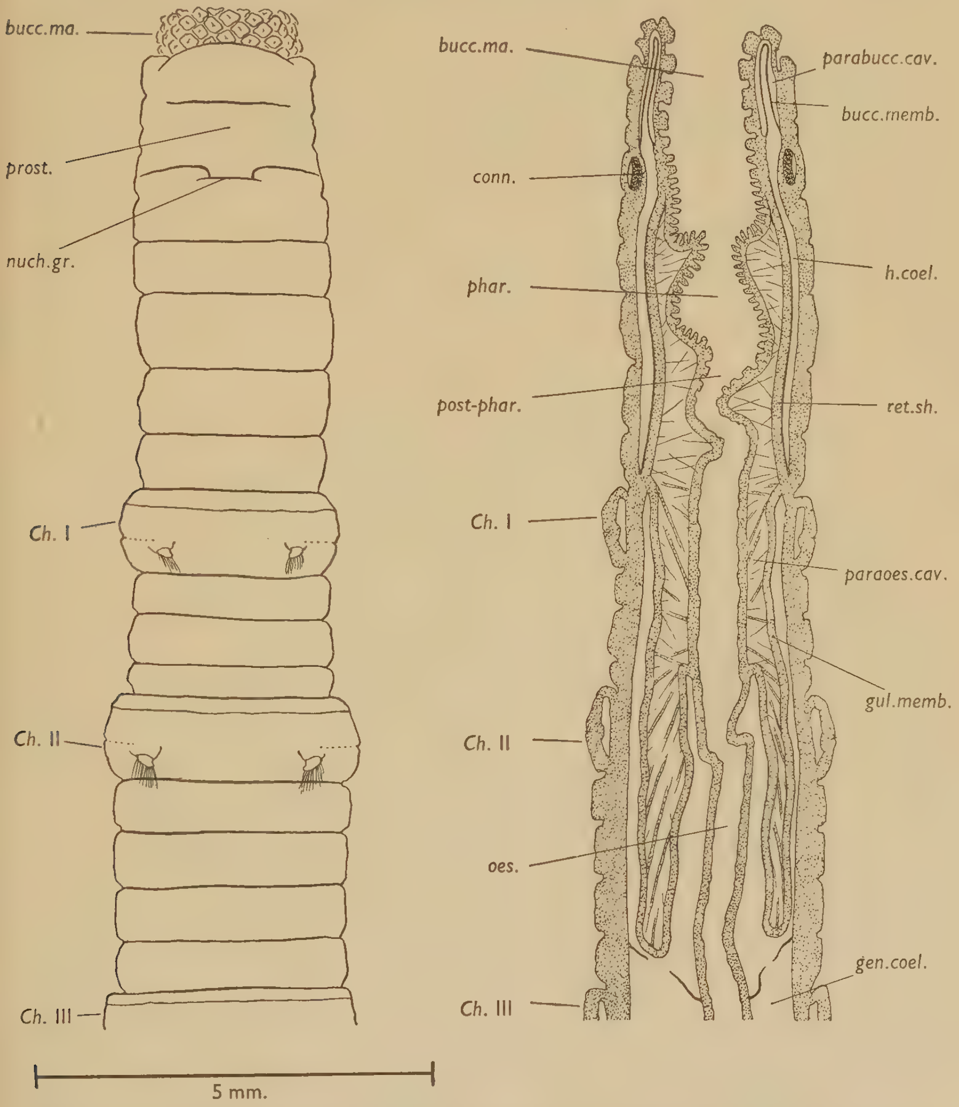
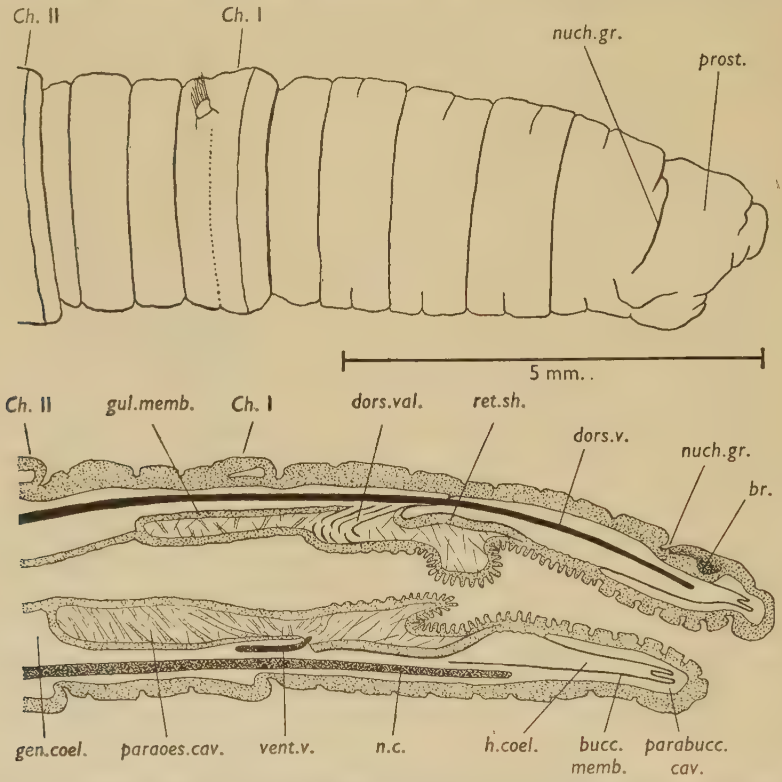
[1] The buccal teeth are more rounded in my Neapolitan specimens than in my Canadian ones. This may be due to the fact that the Neapolitan specimens are much smaller; but Ashworth (1912) has enumerated several other differences between Neapolitan and Pacific members of this species. The statements in my text about the form of the normally extruded proboscis in *claparedii* are based on worms watched at Naples in 1949. I have not seen living Canadian specimens.


bucc.ma.
prost.
nuch.gr.
Ch. I
Ch. II
Ch. III
5 mm.
bucc.ma.
conn.
phar.
post-phar.
Ch. I
Ch. II
oes.
Ch. III
parabucc.cav.
bucc.memb.
h.coel.
ret.sh.
paraoes.cav.
gul.memb.
gen.coel.

Text-fig. 5. Horizontal section through the anterior end of *ecaudata*, and outline of the worm in dorsal aspect. Lettering as on p. 28. (In Text-figs. 5–10, the section was drawn first, from microtome sections or dissections of relaxed specimens, and the surface drawing was then fitted to the same outline.)

The buccal region of *ecaudata* is characterized by the presence of a stout membrane, the buccal membrane (Text-figs. 5, 6; Pl. IV, fig. 3; *bucc.memb.*).

The membrane consists of two very thin epithelial layers with dense connective tissue between them, and separates the head coelome from a smaller cavity, the parabuccal cavity (*parabucc.cav.*). There is apparently no peritoneal lining between the parabuccal cavity and the tissues of the buccal mass, whose interstitial spaces open freely into the parabuccal cavity. The simplest explanation of these facts is to suppose that the buccal membrane represents the peritoneal lining of the head coelome which has separated away from the other tissues of the buccal mass—a modification which would obviously facilitate the movements of the papillae and of the buccal mass as a whole. On this interpretation, the parabuccal cavity is not coelomic, but an extension of the interstitial spaces. The epithelial layer on that face of the buccal membrane which abuts on the head coelome is the true coelomic epithelium and the other is comparable to the endothelial linings of blood vessels and other non-coelomic cavities.



Text-fig. 6. Median sagittal section through the anterior end of *ecaudata*, and outline of the worm in lateral aspect. Lettering as on p. 28.

The buccal membrane meets the gut at the junction of buccal mass and pharynx, and the body wall along the line of the brain and connective nerves.

Mid-ventrally, it extends backwards for a short distance over the ventral nerve cord and then ends abruptly, in such a way as to leave a narrow passage through which the parabuccal cavity communicates with the head coelome (Text-fig. 6).

There is no buccal membrane in *claparedii* or *marina*, and the state of affairs in these species can be derived from that in *ecaudata* by supposing that it has simply disappeared, throwing head coelome and parabuccal cavity together to form a single space. There is no barrier at all between the interstitial spaces of the buccal papillae and the head coelome (Pl. IV, fig. 4). The disappearance of the buccal peritoneum presumably increases the mobility of the papillae in these two species, since they can be inflated or deflated by varying the pressure in the head coelome.

The Gular Membrane

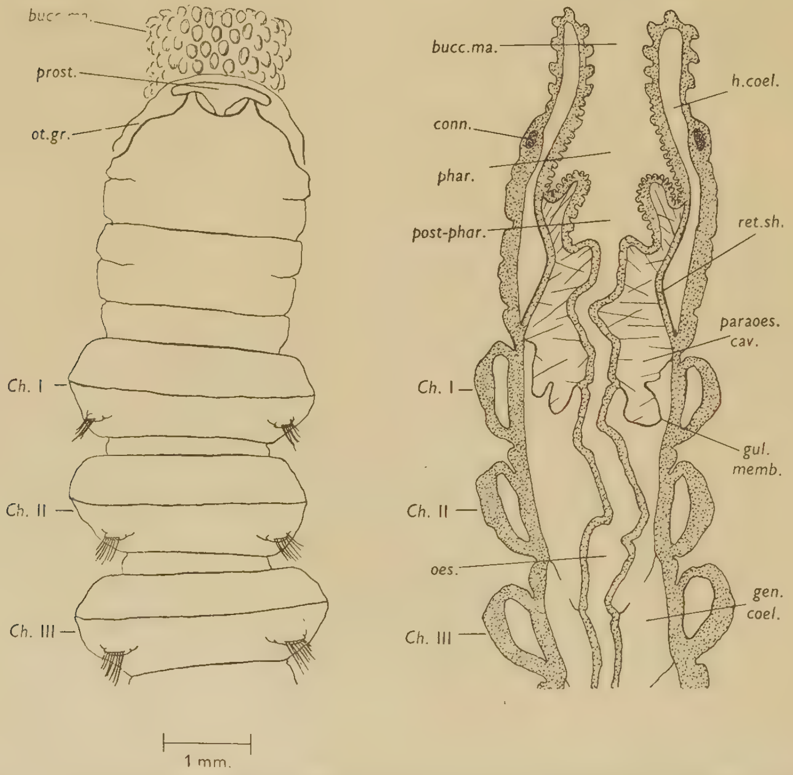
This structure varies very greatly from species to species, in general form and in the strength of its musculature.

In *ecaudata*, the gular membrane reaches its highest degree of development (Text-figs. 5, 6). It is inserted on the oesophagus between the first and second chaetigerous annuli, but it continues farther backwards in the form of two capacious pouches—the septal pouches, in the terminology of Ashworth (1912)—which lie lateral and ventral to the oesophagus and reach nearly or quite as far as the third chaetigerous annulus. Sometimes they even pass beyond this annulus, their tips protruding backwards through the large clefts which exist on each side of the 'second diaphragm'. The musculature of the gular membrane of *ecaudata* differs from that of the other two species in having two layers, a circular lying behind and outside a radial, and each of these layers taken by itself is more massive than the whole musculature of the membrane in *marina* or *claparedii*. There is little doubt that contraction of the gular membrane plays a major part in proboscis extrusion in *ecaudata*.

The dorsal valve of *ecaudata* is narrow and its terminal perforations are not very large, but the muscle strands in its wall are numerous and well developed. On the ventral side, a pair of stout columns of muscle detach themselves from the lateral walls of the ventral foramen, just beside the point of penetration of the ventral vessel into the paraoesophageal cavity, and cross the cavity to reach the ventral side of the oesophagus, a little way behind the post-pharynx; these are the ventral accessory retractors, and are considerably more massive than those of the other species.

The gular membrane of *claparedii* shows the most striking contrast to that of *ecaudata* (Text-figs. 7, 8). It is extremely delicate and transparent, and generally thrown into loose folds. Its musculature consists of a very thin layer of radial fibres. The structure as a whole is evidently too delicate to be of any importance in proboscis activity; it seems, indeed, to be a vestigial organ. There are no septal pouches.

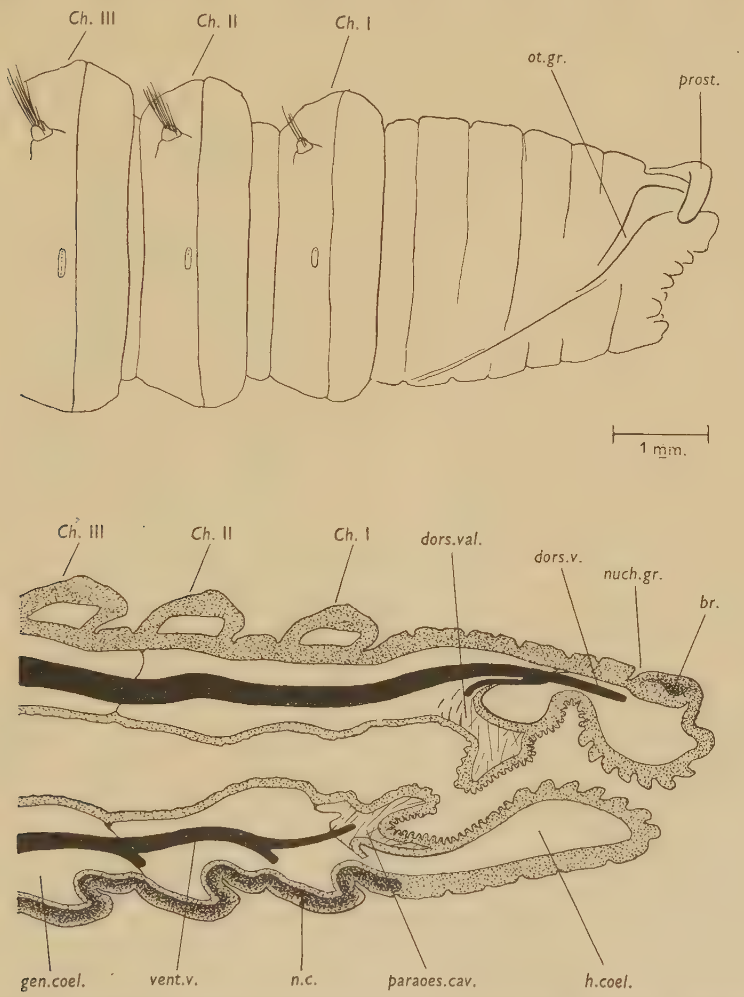
Along the mid-dorsal line, the gular membrane of *claparedii* adheres to the gut wall (contrast Text-figs. 6 and 8). The dorsal valve is widely perforated. These two features together suggest that body fluid driven forwards from the hinder segments could enter the paraoesophageal cavity more readily in *claparedii* than in *ecaudata*.



Text-fig. 7. Horizontal section through the anterior end of *claparedii* (Neapolitan specimen) and outline of the worm in dorsal aspect. Lettering as on p. 28.

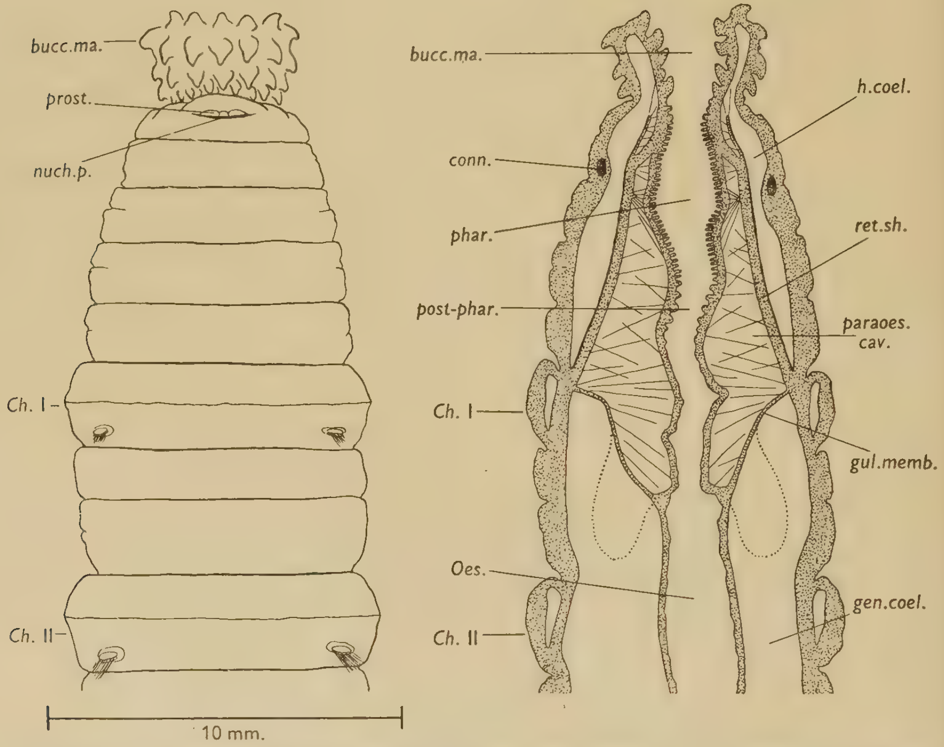
The gular membrane of *marina* is intermediate in its degree of development between those of the other two species. It has the general form of a rather flat cone with the apex directed backwards (in the relaxed worm), and it continues posteriorly into a pair of small septal pouches below the oesophagus (Text-figs. 9, 10). At the point where it meets the oesophagus, the circular muscle layer of the latter forms a conspicuous sphincter. The musculature of the membrane is radial only, but considerably more powerful than that of *claparedii*.

The gular membrane of *marina* is bound closely to the oesophagus by radial strands in the mid-dorsal line. The dorsal valve is more widely perforated than in *ecaudata*. Forwardly moving fluid in the general coelome could easily enter the paraoesophageal cavity through the dorsal valve.


Ch. III
Ch. II
Ch. I
ot.gr.
prost.
1 mm.
Ch. III
Ch. II
Ch. I
dors.val.
dors.v.
nuch.gr.
br.
gen.coel.
vent.v.
n.c.
paraoes.cav.
h.coel.

Text-fig. 8. Median sagittal section through the anterior end of *claparedii* (Neapolitan specimen) and outline of the worm in lateral aspect. Lettering as on p. 28.

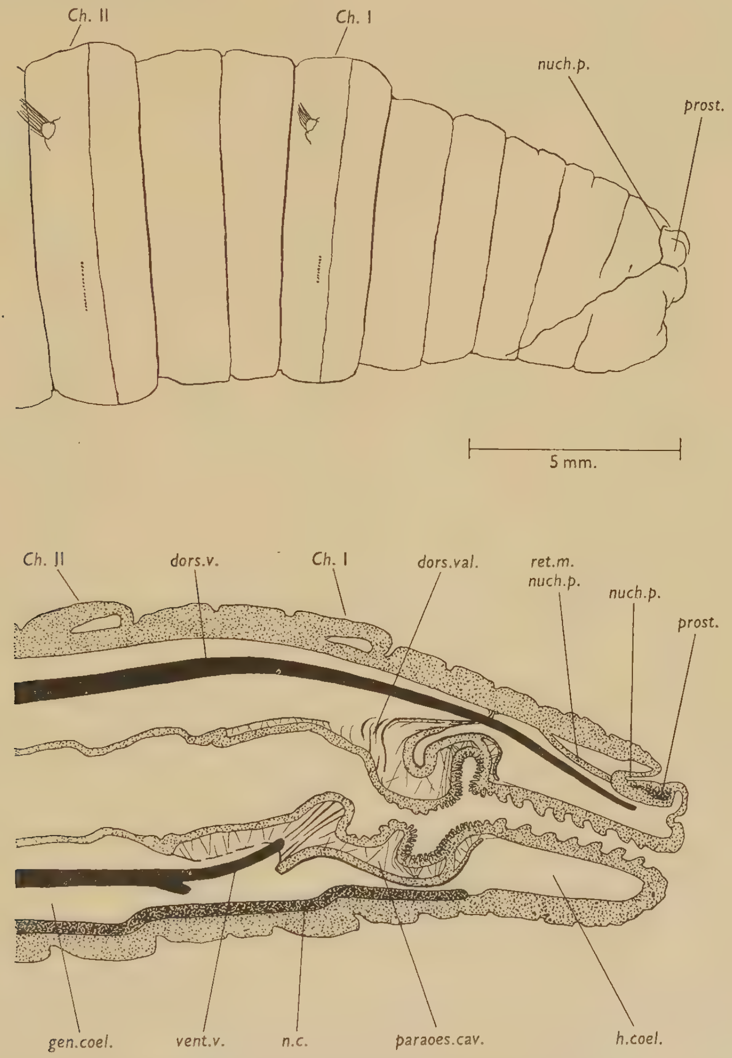
There is also a second entry point on the ventral side in *marina*. As the ventral vessel runs forwards to its point of entry into the paraoesophageal cavity, it is arched over by the floor of the latter (Text-fig. 10). The arch has, in transverse section, roughly the form of a V with the apex upwards. The lateral walls of the V contain numerous muscle strands running to the mid-ventral line of the oesophagus, and their membranous part, though continuous below, is freely perforated between the strands near the apex of the V. The arch may be termed the ventral valve (Pl. III, *vent.val.*). Fluid can easily flow through the perforations from the general coelome into the paraoesophageal cavity, but a high pressure in the latter would tend to press together the imperforate ventral parts of the side walls, and this would obstruct a reverse flow.



Text-fig. 9. Horizontal section through the anterior end of *marina*, and outline of the worm in dorsal aspect. The dotted lines give the position of the septal pouches, which lie ventral to the plane of the section. Lettering as on p. 28.

The Insertion of the Gular Membrane and Retractor Sheath on the Body Wall

The general form of the line of insertion of these two sheets on the body wall is always the same (Text-fig. 2), but its position varies slightly from species to species. In the mid-dorsal line it is farthest forward in *claparedii* and farthest back in *ecaudata* (Text-figs. 6, 8 and 10). Ventrally, immediately beside the ventral foramen, the sheets meet the body wall at the anterior boundary of the first chaetigerous annulus in *ecaudata*, and slightly in front of it in *claparedii*. In *marina* the gular membrane meets the ventral body wall just behind the anterior margin of the first chaetigerous annulus, while the retractor sheath runs back to the hinder half of the annulus next behind the first chaetigerous annulus.


Ch. II
Ch. I
nuch.p.
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5 mm.
Ch. II
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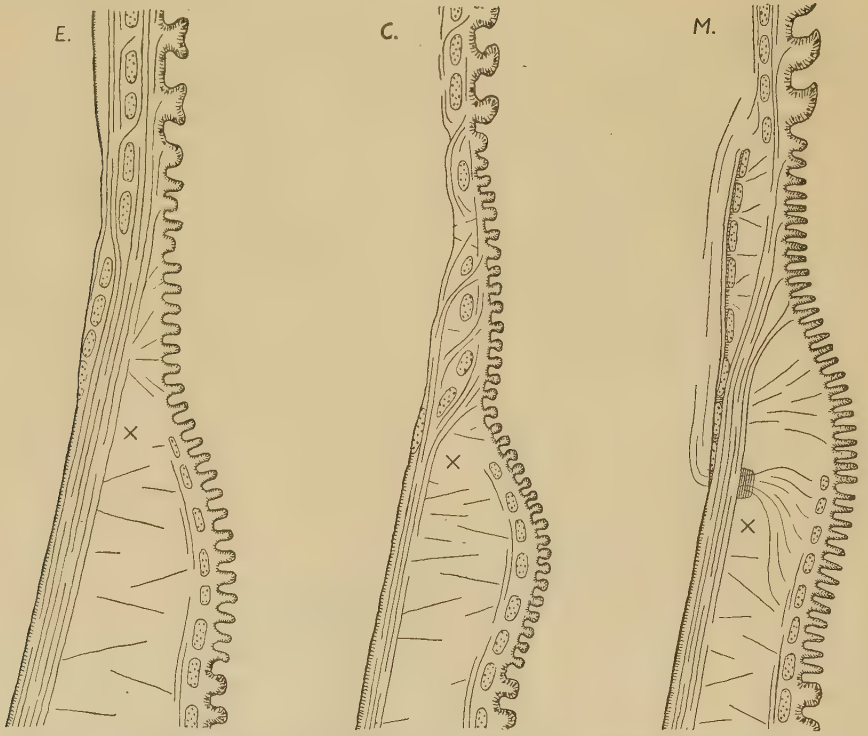
dors.val.
ret.m.
nuch.p.
nuch.p.
prost.
gen.coel.
vent.v.
n.c.
paraoes.cav.
h.coel.

Text-fig. 10. Median sagittal section through the anterior end of *marina*, and outline of the worm in lateral aspect. Lettering as on p. 28.

The extent to which the head coelome and general coelome can communicate between the body wall and the decussation line of the two sheets is also variable. There is always a ventral foramen, and in *ecaudata* this is the only communication. In *claparedii* there are also two large foramina on each side, at the levels of the notopodium and neuropodium. In *marina* there are numerous fine openings round the whole periphery, as can be clearly seen in a well distended specimen. The extent to which these various openings allow fluid to flow between the cavities must be greatly decreased, in any species, if the muscle columns of the two sheets contract in such a way as to pull their decussation against the body wall.

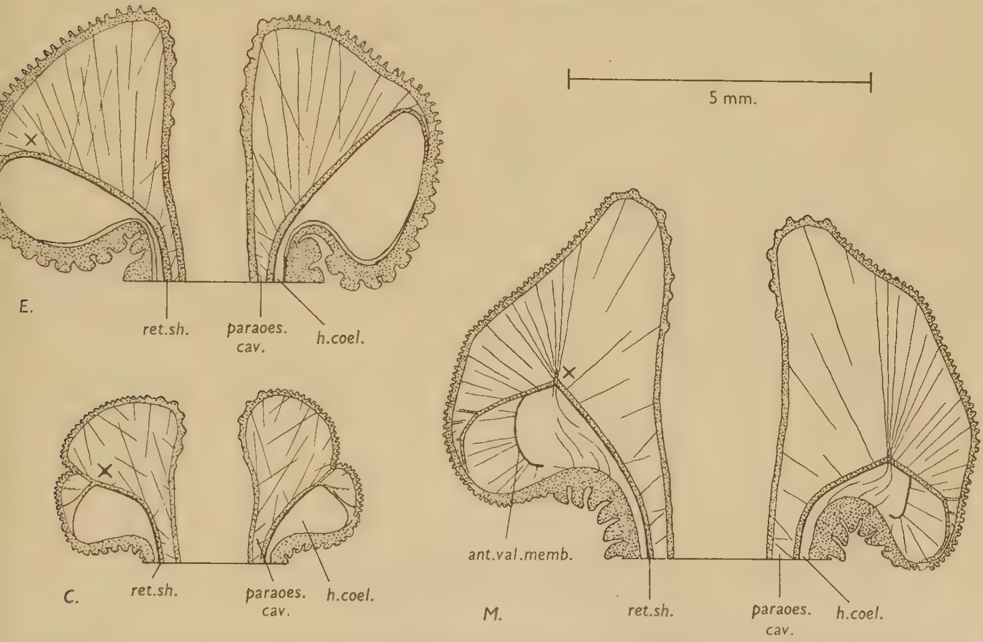


Text-fig. 11. Diagrams of longitudinal sections through the junction of retractor sheath and pharynx in *ecaudata* (*E.*), *claparedii* (*C.*) and *marina* (*M.*). Proboscis withdrawn. The point of junction is marked *X*. Compare Text-fig. 1, p. 3.

The Union of Retractor Sheath and Pharynx

The essential features of this important region are drawn diagrammatically in Text-fig. 11 for *ecaudata* (*E.*), *claparedii* (*C.*) and *marina* (*M.*). The following points are common to all three drawings. The aboral end of the buccal mass is shown above, its interstitial spaces being separated from the head coelome by the buccal membrane in *ecaudata* but not in the other species; then follow the pharynx and the oral end of the post-pharynx below. At the lower end of the drawing, the retractor sheath is seen approaching the gut; it consists of coelomic epithelium on the left, connective tissue (cross-shaded) and muscle. The oral end of the paraoesophageal cavity lies between retractor sheath and gut. At the point *X*, in each drawing, circular muscle appears on the outside of the retractor sheath. This may be regarded as the true point of arrival of the sheath on the gut wall, from whose circular layer the fibres in question are presumably derived.

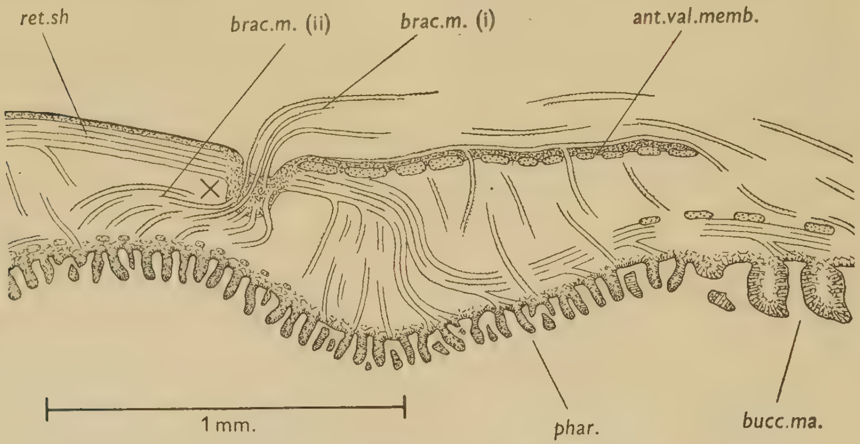
In *ecaudata* the retractor sheath musculature is separated from the gut lining for some distance beyond *X* by a forward continuation of the paraoesophageal cavity, crossed by numerous radial strands, which extends nearly to the buccal mass. The tissues of the junction of pharynx and buccal mass are compactly put together, and there is little or no possibility of a forward flow of fluid out of the oral end of the paraoesophageal cavity. The positions assumed by the various components in the extruded proboscis are shown in Text-fig. 12*E.*



Text-fig. 12. Sections through the extruded proboscis of *ecaudata* (*E.*), *claparedii* (*C.*) and *marina* (*M.*). The position of *X* is the same as in Text-fig. 11. Lettering as on p. 28.

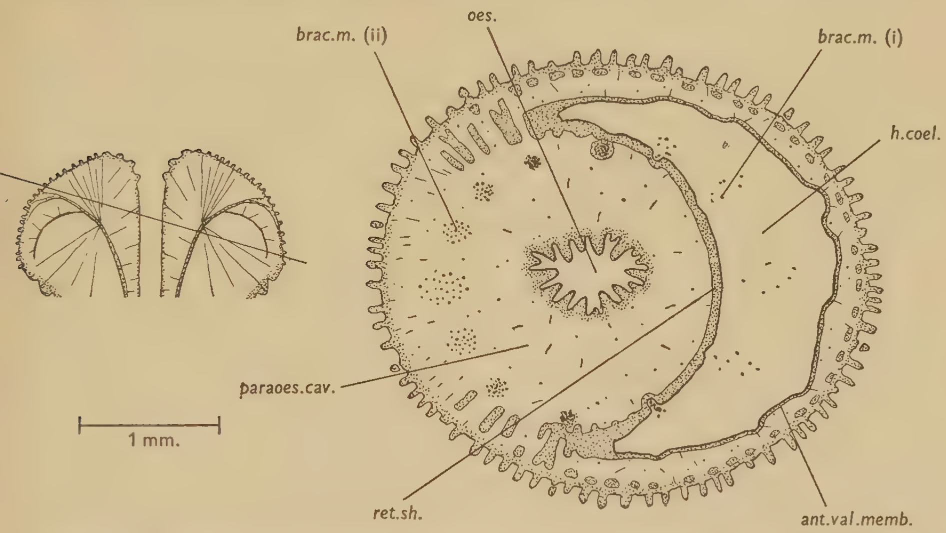
In *claparedii* the bulk of the retractor sheath musculature crosses to the gut lining immediately oral to *X* (Text-fig. 11 *C.*). This is the cause of the 'waist-line' seen in the distended pharynx (Text-figs. 3 *C.*, 12 *C.*). The muscle columns constitute a nearly, but not quite, complete partition at this level. On its oral side the tissues of the pharynx are very loose, with extensive interstitial spaces; they are cut off from the head coelome by a delicate but imperforate membrane, presumably peritoneal, which is bound to the gut lining by radial strands, and extends forwards as far as the junction of pharynx and buccal mass. Here the tissues are tightly bound together, so there is little or no possibility of a forward escape of fluid from the paraoesophageal cavity.

It is in *marina* that the most remarkable specializations of this region are found. The breakdown of the buccal peritoneum extends backwards as far as *X*, and the wall of the oral half of the pharynx is greatly modified to form a structure which will be termed the anterior valve (Text-fig. 13). The most characteristic feature of the valve is the anterior valve membrane (*ant.val.memb.*): this is a continuous membrane encircling the gut and ending anteriorly in a sharp but rather irregular boundary at the level where the pharynx meets the buccal mass. It consists of the following layers: (i) a very thin layer of longitudinal muscle, on the side towards the head coelome; (ii) connective tissue; (iii) circular muscle, on the side towards the gut lining. The anterior valve membrane is evidently a part of the gut wall which has split away from the rest. Beneath it there lies a clear space crossed by radial strands; then come the remaining layers of the gut wall (more of the circular muscle, then superficial longitudinal muscle, connective tissue and epithelium). The retractor sheath muscles cross this space (to become the superficial longitudinal layer) as a series of discrete, parallel columns. Fluid can easily flow forwards from the oral end of the paraoesophageal cavity, passing between these columns and under the anterior valve membrane, to reach the head coelome. The valve membrane presumably blocks any reverse flow, at least when the proboscis is withdrawn (Pl. IV, fig. 5).



Text-fig. 13. Longitudinal section through the anterior valve of *marina*. Proboscis withdrawn. Lettering as on p. 28.

The muscles of the main (deep) longitudinal layer of the buccal mass run backwards towards the valve as a series of roughly parallel strands. Some of them enter the longitudinal layer of the valve membrane at its free margin (often accompanied by strands of connective tissue) and doubtless serve to hold it in position. Others run outside the membrane—i.e. over its coelomic face—and converge, like pencils of rays in an optical diagram, to focus on certain points which are evenly spaced round the periphery of the valve at its level of junction with the retractor sheath; these strands will be termed the oral bracer muscles (Text-figs. 12–14, *brac.m.* (i)). At the points on which these strands are focused, the retractor sheath muscles are separated by dense plugs of connective tissue, which are generally pulled rather inwards towards the gut lining. One of the plugs is shown in section in Text-fig. 13. A second series of muscle strands, more numerous than the first, radiates from each plug to the wall of the aboral half of the pharynx; these will be termed the aboral bracer muscles (*brac.m.* (ii)). Some of the aboral bracer muscles are direct continuations of the oral ones, but others arise in the plug. The main function of these remarkable muscle strands is probably the holding of the parts in position when the proboscis is extruded (Text-figs. 12, *M.*; 14).



Text-fig. 14. Slightly oblique transverse section through the extruded pharynx of *marina*, to show the relations of the bracer muscles. Lettering as on p. 28.

The configuration of the anterior valve in the distended proboscis is shown in Text-fig. 12, *M.* and Pl. III. There are wide gaps between the parallel retractor muscle columns as they cross from the base of the anterior valve membrane to the gut lining, and also between the margin of the valve membrane and the buccal mass. No obstacle is presented to a flow of fluid from paraoesophageal cavity to head coelome—and possibly none to a reverse flow in the fully distended proboscis.

Functional Evolution in the Proboscis Apparatus

The structural differences between the species are great. The following discussion attempts to explain them in terms of function.

We may assume (i) that the retractor sheath is relaxed at the moment of extrusion, and (ii) that the proboscis is driven out, largely at least, by a forward flow of body fluid. Such a flow could be produced in two ways. The gular membrane could contract, pressing on the fluid in the paraoesophageal cavity—and the body wall of the hinder segments could contract, pressing on the fluid in the general coelome. Contraction of the gular membrane would cause a forward flow in the paraoesophageal cavity, while that of the body wall would have several effects. Thus the coelomic fluid could displace the gular membrane forwards, or enter the paraoesophageal cavity through the dorsal valve (and the ventral valve, in *marina*), or enter the head coelome through the ventral foramen and such other openings as exist between the decussation line and the body wall. We may note in passing that the structure of the 'second and third diaphragms' is such that they would not seriously obstruct a flow of fluid along the body.

The anatomy of *ecaudata* strongly suggests that in this species the gular membrane is the main agent in extrusion. Its capacity is great and its musculature is strong. Its contraction would cause a forward movement of the point of union of retractor sheath and pharynx, for the tissues at this point are compactly put together and would block the passage of fluid. The proboscis would therefore be pushed towards the mouth. The body wall could reinforce this action by raising the pressure in the general coelome. A swift and forcible injection of fluid from the general coelome into the paraoesophageal cavity through the dorsal valve seems unlikely to occur, because the extrusion of the proboscis is a reversible process, and there is no means by which the injected fluid could rapidly escape again in *ecaudata*. Such assistance as the hinder body wall may give is more probably by the application of pressure to the outside of the gular membrane. The function of the dorsal valve may be analogous to that of a trickle charger—to allow the entry of fluid into the paraoesophageal cavity as a replacement for any which has leaked away through the surrounding membranes during normal proboscis activity.

Other factors may play a part in extrusion. For example, contraction of the longitudinal muscles of the body wall of the head would tend to roll the buccal mass forwards and outwards, owing to the incompressibility of the fluid in the head coelome, if there were no simultaneous backward flow through the ventral foramen. It nevertheless seems, from the anatomical relationships, that a forward movement of fluid produced by contraction of the gular membrane musculature is of major importance in *ecaudata*.

The other two species differ from *ecaudata* in the following important respects: (i) they distend the proboscis into a fuller, more spherical form;

(ii) their buccal papillae are teeth, not suckers; (iii) their gular membranes are less extensive and less powerful; (iv) the only communication between the general coelome and head coelome in *ecaudata* is through the ventral foramen, but the other two species have additional communicating channels (through the dorsal, ventral and anterior valves in *marina*; through the large openings between decussation line and body wall at the levels of the notopodia and neuropodia in *claparedii*); (v) they have lost the buccal peritoneum. The third of these differences suggests that the main responsibility for propulsion has shifted from the gular membrane to the body wall; this conclusion seems certain for *claparedii* and probable for *marina*. The fourth suggests that the point at which the raised pressure is brought to bear has also shifted. In *ecaudata* we supposed a forward movement of fluid in the paraoesophageal cavity, blocked by and therefore displacing the union of retractor sheath and pharynx. In *marina* the development of the anterior valve completely alters the picture; it seems likely that the forwardly moving body fluid would flow through the valve and press on the advancing tip of the buccal mass. In *claparedii* a rise in the pressure could cause a forward displacement of the delicate gular membrane or a flow through the openings between decussation line and body wall; it could therefore be brought to bear both on the pharynx and on the buccal mass. The fifth difference allows the body fluid to press unhindered into the central cavities of the buccal teeth.

These considerations suggest a co-ordinated evolutionary picture. The supersession of the gular membrane by the body wall means that the proboscis machine is now driven by a motor of much greater power and capacity. The shift in the point at which the fluid pressure operates means that the buccal mass can be used more effectively as a rasp.

The above remarks are, however, speculative. Our knowledge of the movements which the living proboscis performs is still very incomplete, and any theories of its mechanism are therefore insecure. There are differences of habitat between the species: *marina* and *claparedii* are chiefly found on sandy and muddy beaches, while *ecaudata* lives among stones and rocks; but whether there are corresponding differences in the uses to which the proboscis is put remains to be seen. Perhaps, when fuller information about the living worms is available, we shall understand why one species has suckers, and another teeth, on the buccal mass, and this may give us the key to their internal modifications.

SUMMARY

The anatomy of the structures responsible for proboscis activity is described in detail in *Arenicola ecaudata* Johnston, *A. claparedii* Levinsen and *A. marina* L.

The proboscis apparatus includes the following components: the proboscis itself (i.e. the eversible part of the gut), the first part of the oesophagus, the

retractor sheath, and the gular membrane ('first septum' or 'first diaphragm' of previous authors). The retractor sheath and gular membrane are two muscular sheets inserted on the body wall at the same level; the retractor sheath runs to the proboscis and the gular membrane to the oesophagus (Text-figs. 1, 2; pp. 3, 6). They divide the space between gut and body wall into three distinct cavities, which communicate with each other at certain special points only, as described in the text. These cavities are the head coelome, in front of the retractor sheath, the paraoesophageal cavity between the two sheets, and the general coelome behind the gular membrane. The paraoesophageal cavity is crossed by numerous fine muscle strands, running from the two sheets to the gut.

The suggestion is made that the retractor sheath and gular membrane are both derived from the first septum. A cleft has appeared in the septum, splitting it into the two sheets; the cleft becomes the paraoesophageal cavity.

The papillae of the buccal mass (the oral end of the proboscis) are sucker-shaped in *ecaudata* but conical teeth in *claparedii* and *marina*. The papillae are hollow, due to the great development of their interstitial spaces. In *claparedii* and *marina*, but not in *ecaudata*, the buccal peritoneum has broken down, so that coelomic fluid in the head coelome can directly enter and distend the papillae.

The gular membrane of *ecaudata* is very extensive and thrown backwards into a pair of large septal pouches latero-ventral to the oesophagus (Text-figs. 5, 6; pp. 13, 14). Its musculature is powerful and consists of two layers, radial and circular; in the other two species the musculature is radial only. Appearances suggest that the contraction of the gular membrane plays a major part in proboscis extrusion in *ecaudata*. Such contraction would cause a forward movement of fluid in the paraoesophageal cavity; this would press against and displace the junction of retractor sheath and proboscis and so drive the proboscis forwards.

The gular membrane of *claparedii* is extremely delicate and lacks septal pouches; it appears to be a vestigial organ (Text-figs. 7, 8; pp. 16, 17). Proboscis extrusion in this species is probably due to a forward movement of fluid driven by the muscles of the body wall. This fluid could displace the gular membrane and so cause a forward pressure on the junction of retractor sheath and proboscis. It could also flow into the head coelome, through five wide openings between the body wall and the junction of gular membrane and retractor sheath, and so bring pressure to bear on the teeth of the emerging buccal mass.

The gular membrane of *marina* is intermediate in strength between that of *ecaudata* and that of *claparedii*; it has a pair of small septal pouches (Text-figs. 9, 10; pp. 18, 19). The proboscis is probably driven out partly by the gular membrane and partly by the body wall. Fluid can pass from the general coelome into the paraoesophageal cavity through two openings in the median plane, the dorsal and ventral valves. The junction of retractor sheath and proboscis is elaborately modified in *marina* to form the anterior valve, through which fluid can flow from the paraoesophageal cavity into the head coelome (Text-fig. 13, p. 22). These modifications probably mean that the main pressure of the forwardly moving body fluid is brought to bear on the teeth of the advancing buccal mass.

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EXPLANATION OF PLATES

Plate I

Lateral views of the anterior ends of three artificially distended specimens, to show how the form of the proboscis differs from species to species.

Fig. 1. *Arenicola ecaudata.* The hollow to the left is due to caving-in of part of the pharynx after preservation. The mouth is just beyond the left-hand boundary. The buccal mass and transition zone are fully exposed.

Fig. 2. *A. claparedii* (Canadian specimen). The buccal mass is concealed by the pharynx, but the transition zone is clearly seen on the right of the latter.

Fig. 3. *A. marina.* Part of the buccal mass is seen ventrally, in a rather abnormal position due to the distension.

Plate II

Figs. 1, 2. Lateral views of the anterior ends of relaxed specimens of *A. ecaudata* (fig. 1) and *A. marina* (fig. 2). The buccal mass is partly extruded in each. Note the contrast in form of the buccal papillae.

Fig. 3. Dorsal half of a horizontally bisected specimen of *A. marina.* Proboscis withdrawn. Compare Text-figs. 1 and 9, pp. 3 and 18.

Fig. 4. Horizontal section through the anterior segments of a 1·5 mm. post-larval *A. cristata.* Lettering as on p. 28.

Plate III

Dissection from the right side of a distended specimen of *Arenicola marina* (same specimen as in Pl. I, fig. 3). An explanatory drawing is placed below the photograph. The dissection has been carried nearly, but not quite, to the median plane. The anterior part of the oesophagus and the tip of the dorsal valve have been removed altogether, to show the internal structure of the proboscis; then follows an area over which the right lateral wall of the oesophagus has been removed (cross shading in the drawing). The ventral edge of the left septal pouch is visible in the angle between ventral vessel, ventral mesentery and gular membrane (dark stippling in the drawing). Lettering as below. Compare Text-figs. 10 and 12, *M.*, pp. 19 and 21.

Plate IV

Figs. 1, 2. Longitudinal sections through single buccal papillae of *A. ecaudata* (fig. 1) and *marina* (fig. 2). Both worms are relaxed, with the proboscis withdrawn. The buccal papilla of *ecaudata* is sucker-shaped, with the top depressed; its internal musculature is clearly shown. That of *marina* is tooth-shaped (oral end to the left); a small blood vessel is entering the base of the papilla and there is another in the tip of the 'tooth'.

Figs. 3, 4. Transverse sections including the ventral body wall (below) and the ventral wall of the buccal mass (above) in *ecaudata* (fig. 3) and *marina* (fig. 4). The two sections are as nearly as possible from the same position, i.e. just in front of the bifurcation of the ventral nerve cord. The sucker-shape of the buccal papillae of *ecaudata* is again obvious. The two folds of the buccal membrane of *ecaudata* are seen as thick, rather wavy lines crossing the middle of fig. 3; the narrow space between them is the head coelome (compare Text-figs. 5 and 6, pp. 13, 14). In *marina*, there is no barrier between the head coelome and the interstitial cavities of the buccal papillae.

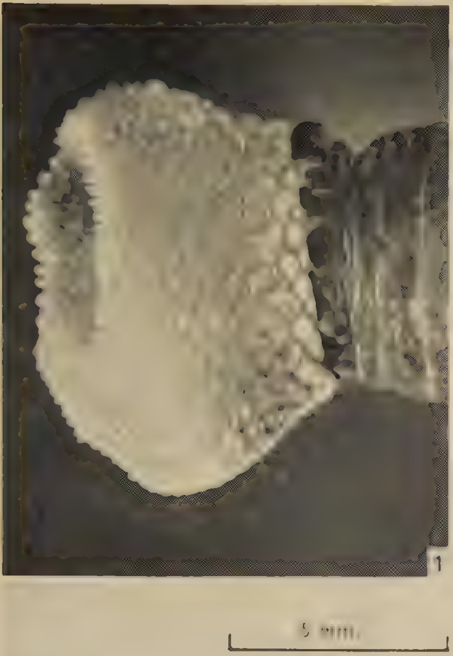
Fig. 5. Longitudinal section through the anterior valve of *marina*. Relaxed specimen; proboscis withdrawn; oral end to the right. The section corresponds to Text-fig. 13, p. 22, except that it does not happen to pass through a bracer muscle perforation. The pale grey object on the upper margin of the photograph, about half-way along, is a longitudinally-running blood vessel; a group of bracer muscle strands can be seen just below it.

Pl. II, fig. 3, was taken by the late Mr F. J. Pittock, and is reproduced from the *Journal of Experimental Biology*, Vol. 14, 1937, by kind permission of the Company of Biologists Ltd.

The other photographs were taken for this work by the following: Pl. I, by Miss J. Hubbard; Pl. II, figs. 1 and 2, and Pl. III, by Mr J. Armstrong; Pl. II, fig. 4, and Pl. IV, by Mr W. Brackenbury.

List of Abbreviations used in the Text-Figures and Plates

ant.val.memb.	anterior valve membrane	*metast.m.*	metastomial muscle
br.	brain	*n.c.*	nerve cord
brac.m. (i)	oral bracer muscles	*nuch.gr.*	nuchal groove
brac.m. (ii)	aboral bracer muscles	*nuch.p.*	nuchal pouch
bucc.ma.	buccal mass	*oes.*	oesophagus
bucc.memb.	buccal membrane	*ot.gr.*	otic groove
Ch.	chaetigerous annulus	*parabucc.cav.*	parabuccal cavity
circ.m.	circular muscle	*paraoes.cav.*	paraoesophageal cavity
conn.	connective nerve	*phar.*	pharynx
D.	diaphragm	*post-phar.*	post-pharynx
D.vest.	diaphragm which becomes vestigial	*prost.*	prostomium
dors.mes.	dorsal mesentery	*ret.m.nuch.p.*	retractor muscle of the nuchal pouch
dors.v.	dorsal vessel	*ret.sh.*	retractor sheath
dors.val.	dorsal valve	*sup.long.m.*	superficial longitudinal muscle
ep. & c.t.	epithelium and connective tissue	*vent.for.*	ventral foramen
gen.coel.	general coelome	*vent.mes.*	ventral mesentery
gul.memb.	gular membrane	*vent.v.*	ventral vessel
h.coel.	head coelome	*vent.val.*	ventral valve
long.m.	longitudinal muscle		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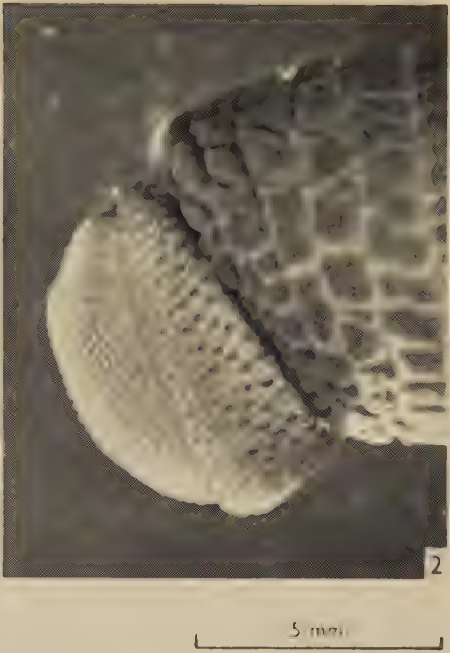
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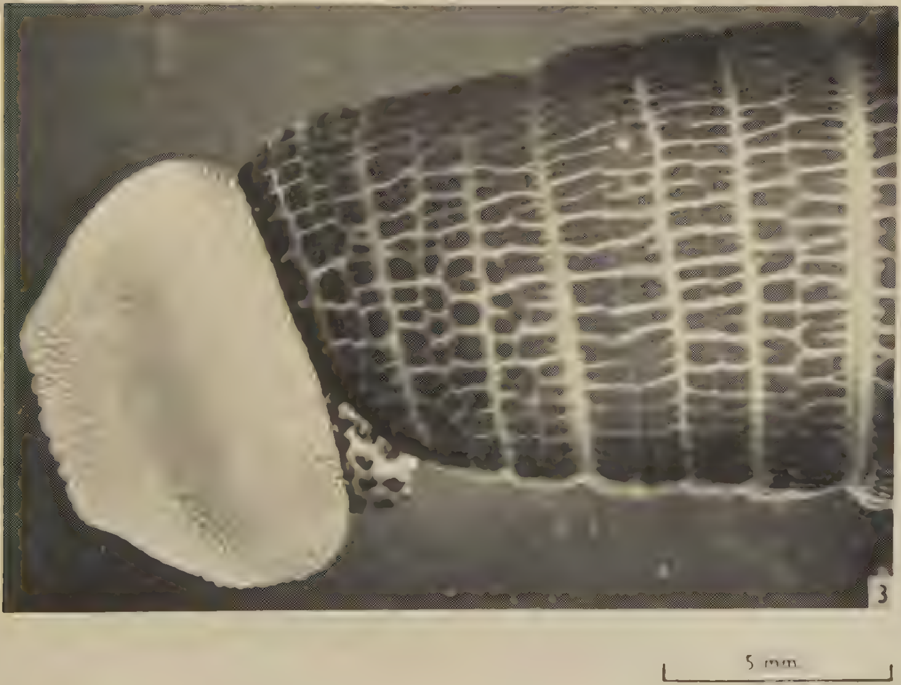
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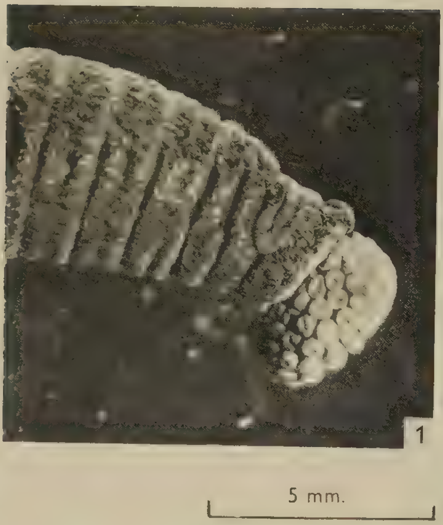
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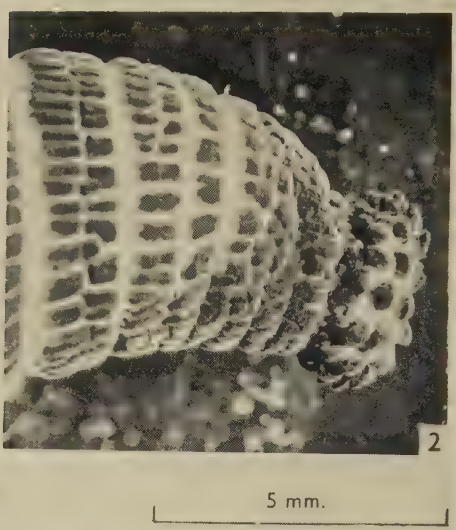
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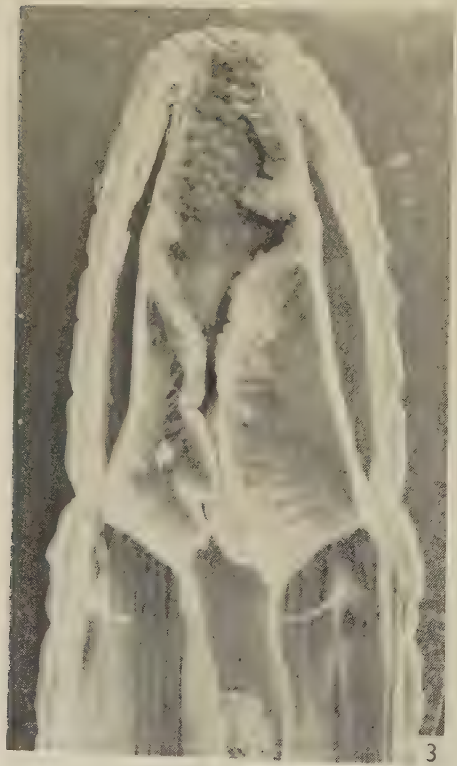
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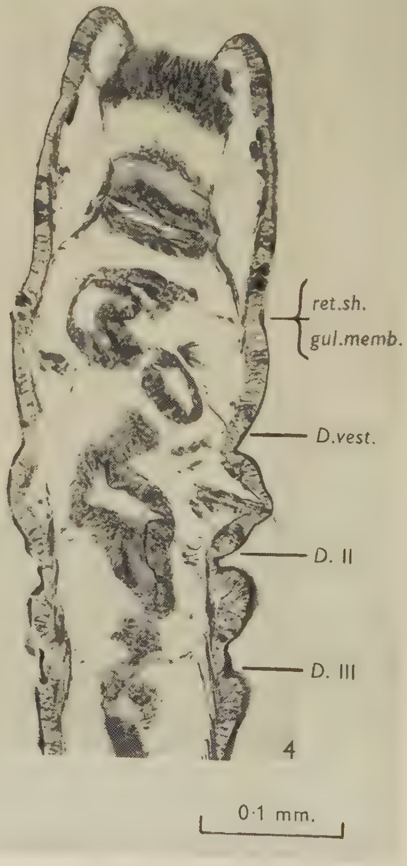
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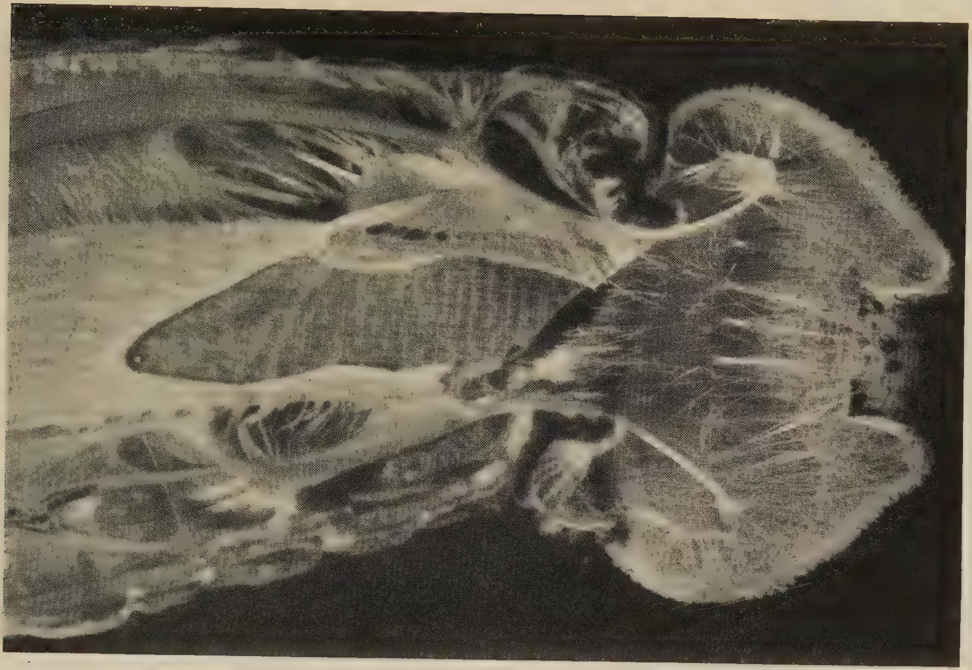

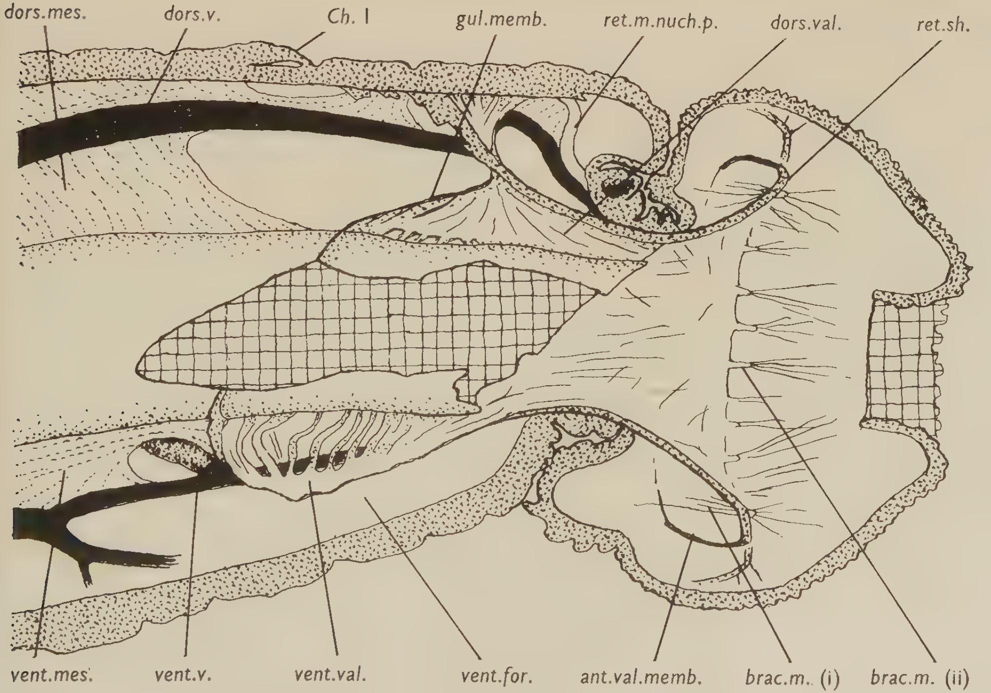
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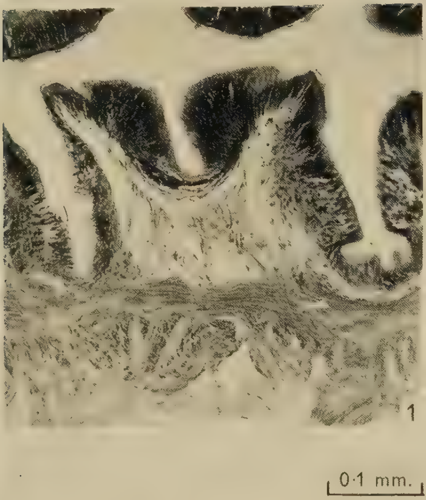
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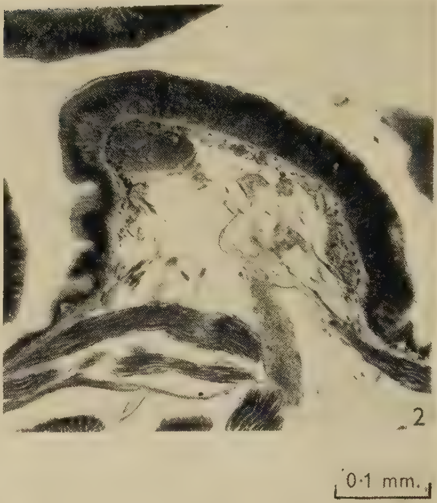
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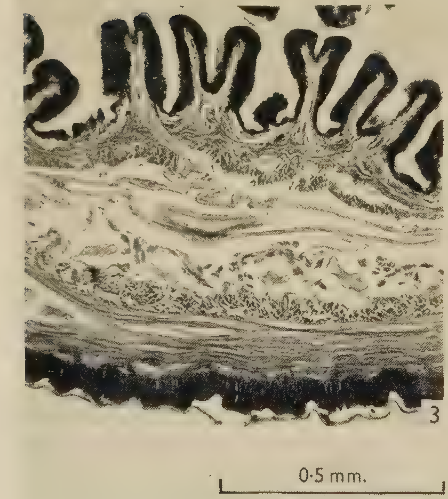
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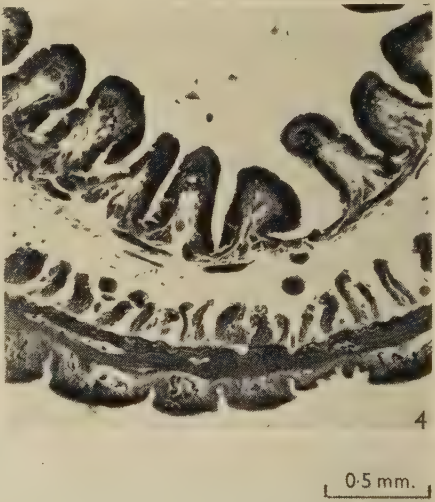
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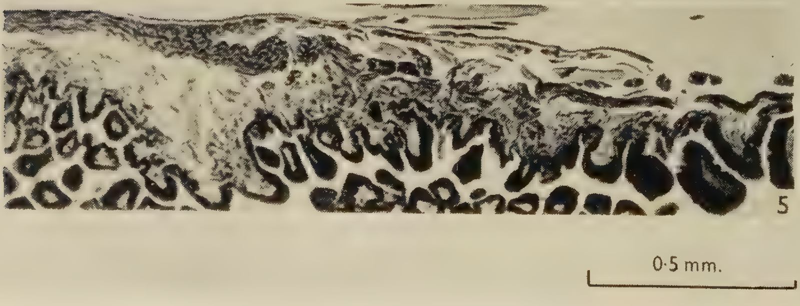
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VARIATIONS IN CHEMICAL COMPOSITION DURING THE DEVELOPMENT OF *HIMANTHALIA ELONGATA* (L.) S. F. GRAY

By Betty Moss
King's College, Newcastle-upon-Tyne

(Text-figs. 1, 2)

Himanthalia elongata (=*H. lorea* (L.) Lyngb.), although a common brown seaweed on parts of the British coast, does not occur in sufficient quantities to render it of commercial importance. As a result, the work of Colin & Ricard (1930) appears to be the only investigation on the chemical composition of this species. Their material was collected in August 1929, but they give no indication of the size or condition of the plants which were analysed.

Seasonal variations in the chemical composition of other brown seaweeds have been studied by various workers (e.g. Lapicque, 1919; Lunde, 1940; Black, 1948 *a*, *b*, 1949, 1950 *a*). Also Black (1950 *b*) has considered the influence of habitat and depth of immersion on the composition of three species of *Laminaria*. In these investigations no indication is given as to whether young or old plants, or sterile or fertile plants, were analysed.

After it had been shown that marked changes in chemical composition were associated with the development of the fertile receptacles in *Fucus vesiculosus* (Moss, 1950), it appeared that different growth stages of the same species collected in any one season might show variations as great as the seasonal ranges which had been reported for other species. *Himanthalia elongata* collected from two habitats, one on the west coast of Scotland and the other on the north-east coast of England, was selected for this study.

COLLECTION OF MATERIAL

One set of material was obtained from Clachan Sound, where a narrow arm of the Atlantic Ocean separates the Island of Seil from the mainland of Argyllshire. The water here is shallow, with a swift current running through, and *Himanthalia* is abundant growing amongst *Laminaria digitata* and up into the succeeding zone of *Fucus serratus*.

Collections were made on three successive days in April 1949, at low-water spring tides, when the plants were never exposed completely. Fig. 1 shows the stages into which they were sorted for chemical analysis:

Stage 1. Young sporelings up to ½ in. in length.
Stage 2. Young sporelings more than ½ in. in length but still with a tubular structure and no flattening of the apex.

Stage 3. Young plants with flattened apices.

Stage 4. Fully grown vegetative plants with the typical button form.

Stage 5. Buttons with young thongs (i.e. fertile receptacles) up to 10 in. in length.

Stage 6. Buttons with thongs 5–7 ft. in length.

Stage 7. Old buttons with spent receptacles which persist several months after gamete extrusion.

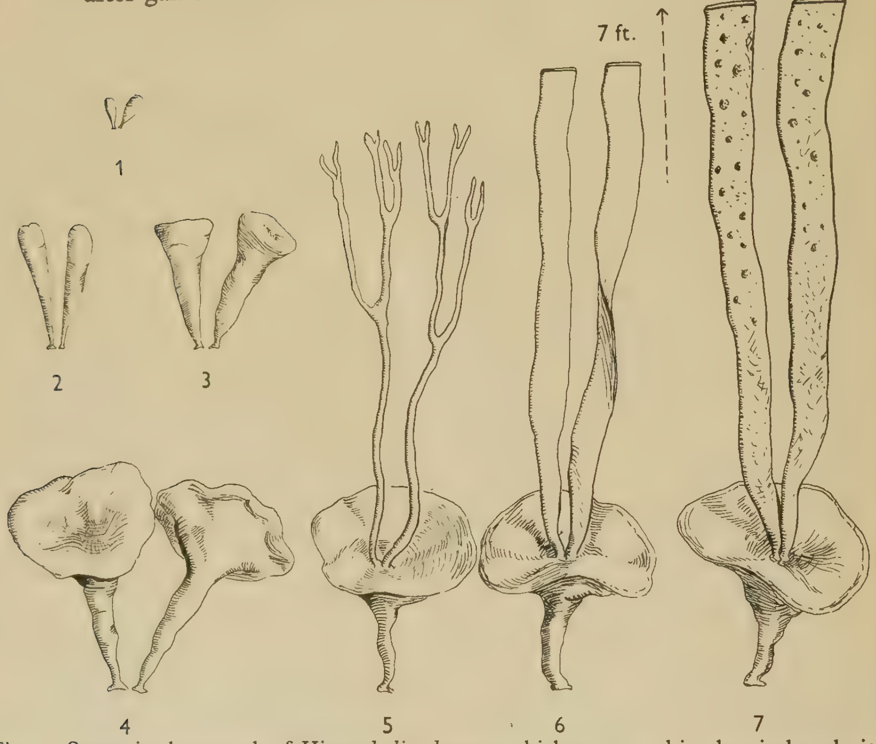

Fig. 1. Stages in the growth of *Himanthalia elongata* which were used in chemical analysis.

In the latter three stages, 5, 6 and 7, the vegetative buttons were cut from the fertile thongs and each part analysed separately, so that variations in chemical composition throughout the development of both the vegetative and fertile tissues could be studied independently.

The following April a similar series of samples were collected from St Mary's Island, Northumberland, where *Himanthalia* grows on exposed ledges, and not in association with *Laminaria* and *Fucus* as in Clachan Sound. Collections were made as the tide receded, and the plants were sorted into similar stages for analysis. However, similar developmental stages of these plants from St Mary's Island were much smaller in size than comparable stages from Clachan Sound.

The analytical methods used are those developed by the Institute of Seaweed Research (Black, 1948*a*; Cameron, Ross & Percival, 1948).

RESULTS

Dry Weight. The percentages of dry matter recorded in Fig. 2A show that an increase occurs from the sporelings to the mature plants from both habitats, but the plants from St Mary's Island always give higher values. A marked increase in dry weight occurs in the old thongs when they become hard and leathery after gamete extrusion. Such an increase was not observed in *Fucus vesiculosus* (Moss, 1950), where the receptacles disintegrate rapidly.

Mineral Ash. Fig. 2B shows the percentage total mineral ash on the anhydrous basis in plants from both habitats, while Fig. 2C gives the water soluble and water insoluble constituents of the ash in plants from Clachan Sound only.

The higher percentage dry matter of plants from St Mary's Island is associated with a lower ash content. The young sporelings from Clachan Sound have an extremely high percentage of mineral ash, 64 %, calculated on the anhydrous basis. This appears to be a higher value than any recorded for a brown seaweed. Black (1948*a*) drew attention to the high percentage of ash, 55 %, in stipes of *Saccorhiza polyschides* (Lightf.) Batt. (= *S. bulbosa* La Pyl.) collected in July 1946. The percentage dry weight of this particular sample was lower than at any other season. In a similar manner, the highest percentage ash is found in the sporelings of *Himanthalia* when their percentage dry matter is lowest.

Crude Proteins. As Fig. 2D shows, there is a gradual increase in crude proteins during the development of the vegetative buttons. The young thongs give high values, but after gamete extrusion there is a marked decrease, until the protein content of the old thongs approximates to that of the senescent buttons.

It is interesting to note that the range in crude proteins from 6·8 to 14·3 % of the dry matter of plants from Clachan Sound is identical with the seasonal range which has been published for *Laminaria digitata* collected from the same habitat (Black 1948*a*).

Mannitol and Laminarin. Fig. 2E shows the percentage mannitol, calculated on the anhydrous basis, in plants from both habitats. There is a marked decrease in the mannitol content of the vegetative buttons as the young thongs begin to grow.

Laminarin was present in very small quantities (less than 2 %) in all samples. The low values of both mannitol and laminarin may be related to the time of the year when the plants were collected, for it has been shown

(Black, 1948*a*, *b*; 1949) that in both *Laminaria* and *Fucus* these substances are lowest during the early part of the year.

Alginic Acid. Variations in the percentage alginic acid calculated on the anhydrous basis are shown in Fig. 2F. This substance is very low in the young

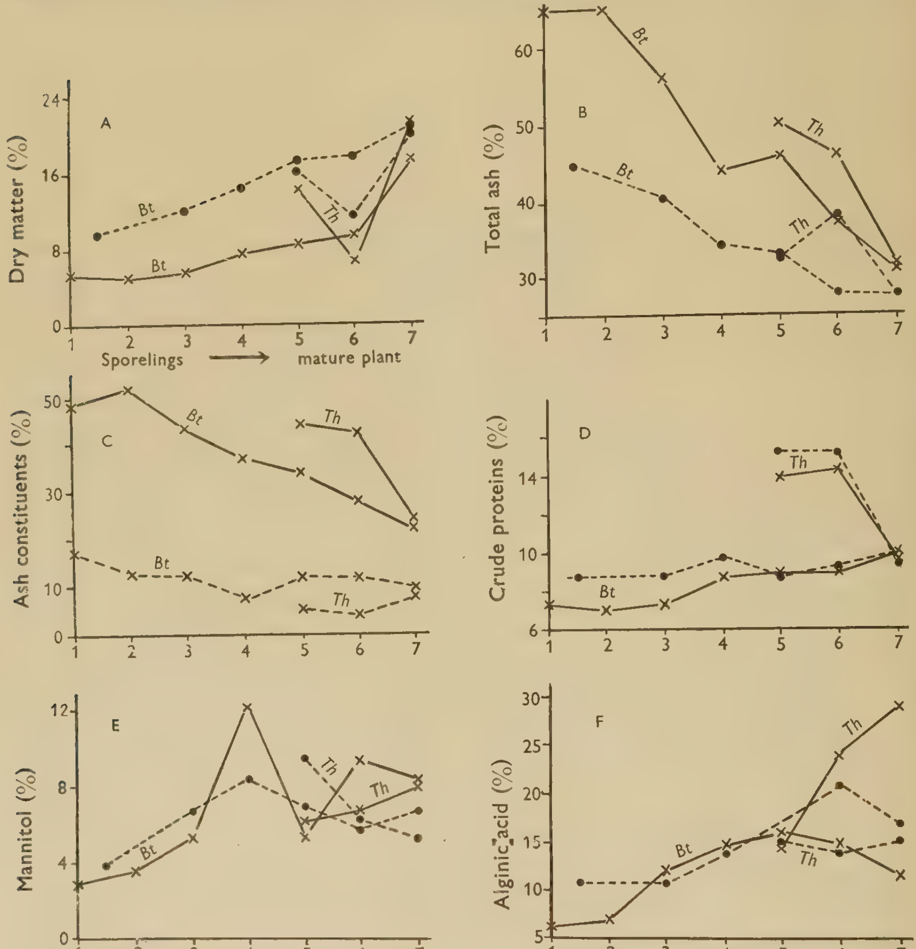

Fig. 2. Variation in chemical composition during development of *Himanthalia elongata*, expressed as percentages of weight. A, dry weight; B, total mineral ash (dry basis); C, water soluble (×——×) and water insoluble (× ---- ×) constituents of the mineral ash (dry basis), of plants from Clachan Sound; D, crude proteins (dry basis); E, mannitol (dry basis); F, alginic acid (dry basis). *Bt*, buttons; *Th*, thongs; ×——×, plants from Clachan Sound; • ---- •, plants from St Mary's Island.

sporelings, especially in those from Clachan Sound. The high value in the very long thongs from this habitat was not recorded in the shorter thongs from St Mary's Island.

DISCUSSION

The young sporelings of *Himanthalia elongata*, with their very low percentage of dry matter, are also characterized by a very high mineral ash and low organic content. These young plants are growing rapidly, and as differentiation proceeds so does the percentage of organic constituents increase. The completion of vegetative activity is then followed by the rapid development of the fertile tissues or thongs. Apart from the noted decrease in mannitol, the growth of the thongs does not influence to any great extent the chemical composition of the buttons, suggesting that there is little or no storage of materials for translocation to the developing thongs.

The young thongs, being lower in mineral ash content and very much higher in proteins, mannitol and alginic acid, have a form of metabolism, as shown by their chemical composition, which differs considerably from that of young vegetative tissues developing at the same season.

While the curves obtained for plants from St Mary's Island follow the same trends as those of plants from Clachan Sound, nevertheless they do show higher percentage dry weights and lower mineral ash contents.

These analyses have shown that during the development of plants of *H. elongata* there may be a range in dry weight of 15 %, in mineral ash of 35 %, in proteins of 7 % and in alginic acid of 20 %. This emphasizes that in *Himanthalia*, as possibly in other brown seaweeds, the stage of development of the plant to be analysed is a major factor to be considered in relation to any variations in chemical composition.

SUMMARY

Chemical analyses have been made of different stages in the growth of *Himanthalia elongata*, which have been collected at the same season from two habitats.

During the development of the vegetative buttons increases in the percentage dry weights, mannitol, crude proteins and alginic acid are found, while mineral ash decreases. The young fertile tissues or thongs are rich in proteins and alginic acid compared with the buttons which produce them.

Smaller plants from St Mary's Island have higher percentage dry weights and lower ash content, while their thongs do not show the high alginic acid content found in larger plants of a similar developmental stage from Clachan Sound.

The range in chemical constituents shown in different growth stages of the same species, collected at the same time from the same habitat, stresses the importance of describing the plant which is analysed when the effects of seasonal or other factors upon the chemical composition of brown seaweeds are being considered.

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THE BIOLOGY OF *ASTERIAS RUBENS* L.
IV. VARIATION IN THE SEX RATIO

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(Text-fig. 1)

There are few records in the literature of the relative proportions of the sexes in natural populations of echinoderms, and the samples studied have mostly been small. Selenka (1867) has stated that in Holothurians in general males are rarer than females, and Becher (1907) found that males were very rare in *Rhabdomolgus ruber*. Koehler (1907) noted that in the ophiuroid *Ophiacantha vivipara* there were more females than males.

Among the echinoids records of sex ratios are more numerous, but the samples used were mostly taken over a short period of the year. In a group of *Centechinus setosus* from Suez there were 400 males and 370 females, and in two samples of *Strongylocentrotus lividus* from Alexandria there were 291 males : 259 females and 238 males : 266 females (Fox, 1924). In a sample of 358 *Echinocardium cordatum* from Port Erin, Moore (1935) found 181 males : 177 females. Ikeda (1931) has recorded a sex ratio approaching equality (100 : 101·8) in the Japanese echinoid *Temnopleurus toreumaticus*. The most valuable contribution to these records is the work of Neefs (1938) on *Strongylocentrotus lividus* from Roscoff. She found that the sex ratio in this species varied from month to month. The percentages of females in her monthly samples, taken over more than one year, were: January, 26·5 and 1; February, 44; March, 45·5; April, 47 and 57; May, 40 and 50; June, 41 and 53; July, 46; August, 46 and 51; September, 62 and 56; October, 62; November, 53, 50·5 and 51; December, 41 and 45. These figures show clearly a preponderance of females in September, October and November. The reason for these fluctuations is not clear although it is possible that they may be connected with the hermaphroditism found in this species (Neefs, 1937).

Pelseneer (1926, p. 157) found that in a sample of 400 *Asterias rubens* taken at Wimereux in summer 1924 there were 184 males and 216 females.

SEX RATIOS IN MEDIUM AND LARGE STARFISHES

In a recent study of large numbers of *Asterias rubens* from four populations in the Plymouth area samples taken throughout the year have yielded further data on the sex ratio in this species. An account of the growth, reproduction and situation of these populations has already been given (Vevers, 1949), and additional material has been collected since then.

The percentage of females in each population, as given in Table I, has been calculated from the total number of medium and large starfishes of each sex caught by otter trawl on the grounds at all times of the year, during the period 1947–50.

It has already been shown that the mean body size of *A. rubens* caught by the otter trawl is larger at E1 and in the Outer Grounds than it is in the Rame-Eddystone Grounds and Plymouth Sound (Vevers, 1949). It will be seen from Table I that populations with larger starfishes have an excess of females over males (Outer Grounds and E1 area). In populations with medium-sized starfishes, as in Plymouth Sound and the Rame-Eddystone Grounds, the sexes are almost equal in number with a slight preponderance of females.

TABLE I

	Mean radius (cm.)	Percentage of females in population	No. of samples	Total in all samples
Rame mud (Agassiz trawl)	3·11	45·6	2	187
Plymouth Sound	9·40	53·6	5	266
Rame-Eddystone Grounds	10·48	51·3	23	1066
Outer Grounds	15·47	55·1	32	2337
E1 area	19·61	70·3	6	336

TABLE II. ANALYSIS OF HAULS OF *ASTERIAS RUBENS* ON RAME MUD IN 1949

(Measurements in centimetres.)

		Males			Females			Percentage of females in sample
Date	Total in catch	No.	Range	Mean	No.	Range	Mean	
12. v. 49	103	57	1–5·5	3·15	46	1–6·0	3·20	44·7
1. vi. 49	84	45	2–6·0	3·09	39	1–6·0	3·01	46·4

SEX RATIO IN SMALL-SIZED STARFISHES

It is not possible to obtain representative samples of small-sized starfishes (radius less than 6 cm.) with the otter trawl, but on three occasions they have been caught in sufficiently large numbers by Agassiz trawl. All these catches were taken on or near the Rame mud area, south-west of Plymouth Sound. The first catch of ninety-five starfishes taken in April 1948 was not sexed, but the other two catches have been satisfactorily sexed and their radius lengths recorded (Table II).

These analyses show that in this population of small starfishes the males tend to be more numerous than the females.

SEASONAL VARIATIONS IN THE SEX RATIO

As the number of samples is relatively large the percentage of females in each catch from the Outer Grounds and Rame-Eddystone populations can be separated, according to season of capture, into six equal (two-monthly) periods covering the year (Table III and Fig. 1). It is then found that in these large and medium-sized starfishes the proportion of females is below 50% during the winter months (November–February), but rises to 55% and even more during the spring and summer.

TABLE III. PERCENTAGE OF FEMALES IN THE OUTER GROUNDS AND RAME-EDDYSTONE GROUNDS POPULATIONS AT DIFFERENT TIMES OF THE YEAR

	Outer Grounds	Rame-Eddystone Grounds
January–February	48·3	46·6
March–April	57·1	54·6
May–June	57·1	54·9
July–August	53·9	54·8
September–October	51·9	52·5
November–December	49·4	47·6

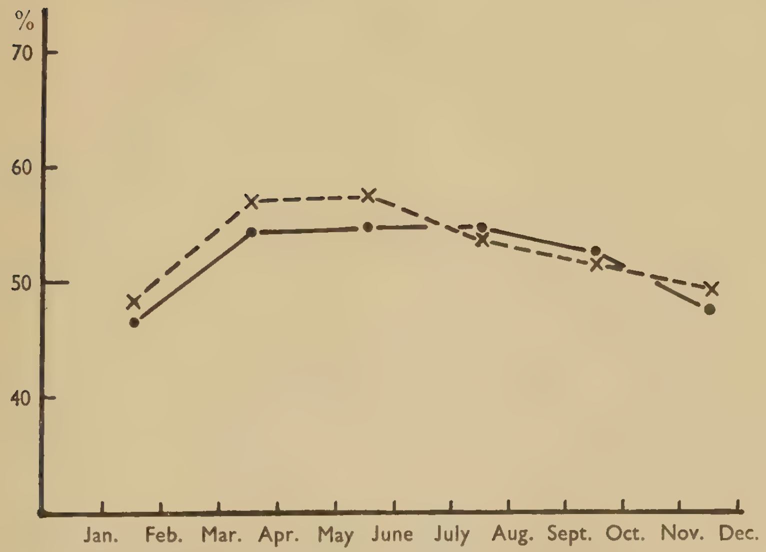

Fig. 1. Percentage of females in two populations at different times of year. ● Rame-Eddystone Grounds; × Outer Grounds.

There are insufficient data for this analysis to be carried out on the samples from the E 1 area, Plymouth Sound and Rame Mud populations.

The curves in Fig. 1 are similar, with that for the Outer Grounds showing a higher percentage of females, at any rate during the spring and early summer.

DISCUSSION

Pelseneer (1926) has shown that in animals in general the males are more numerous at birth or metamorphosis while females are more numerous among the adults of a species. He considered that males were probably a little weaker in constitution and died earlier in life than females. He gave examples which showed that, in some molluscs, insects, fishes, birds and mammals the male is more liable to parasitization than the female.

From the present data on *Asterias rubens* it is apparent that in starfishes over about 6 cm. radius the population samples tend to have relatively more females as the mean body size of the individuals increases.

Seasonal variation in the sex ratio of a population of animals may be associated with selective swarming of one sex, with protandrous hermaphroditism of the type found in *Asterina gibbosa*, with parthenogenesis as in aphids, or with a greater mortality rate in one sex. In *Asterias rubens* there is no evidence of hermaphroditism as a generally occurring phenomenon. Over 4000 specimens of this species have been examined to determine body size and the state of gonad development, and there has been no instance of a gonad showing traces of a change in the nature of its sexual elements.

Kirk (1912) has described a sample of thirty specimens of the crinoid *Actinometra japonica* from one locality in which there were no males, and the females all contained nearly ripe eggs. He considered that this was a case of swarming due to sexual activity, and that it might account for the large isolated masses of the fossil *Uintacrinus* which are often found, pointing out that such a large crinoid with an elaborate system of plankton-gathering arms could not normally live and feed in such dense masses.

At first sight the seasonal change in sex ratio in *Asterias rubens* might appear to be due to a selective swarming of females on the breeding grounds. However, if this happened, and the lacking males still survived, it is difficult to account for their absence from trawl hauls taken over such large uniform areas. It is scarcely possible that large and medium-sized males could hide on an even, flat sandy bottom of the type found on the Outer Grounds, the source of all the larger catches. Nor is there any evidence for long distance movements of starfishes.

There is, however, a possibility of a differential mortality, due to an inherent weakness in the male or to infection by parasites. Although there is no direct evidence of a difference in viability of adult males and females, it has been observed that when a large catch of *A. rubens* (taken by otter trawl) is brought into the laboratory the mortality during the first 2 days of captivity is invariably higher in the males than in the females. This suggests that the males may be more susceptible to the bruising of the ciliated epithelium and other organs which is inevitably suffered in the cod end of the otter trawl.

It is also possible that a differential rate of mortality between the sexes is related to the occurrence in the testes only of infestations of the ciliate parasite *Orchitophrya stellarum* (Vevers, 1951). Not only does this parasite cause complete atrophy of the testes, but it has also been observed that many infected males show very weak carotenoid pigmentation of the aboral integument and a general flabbiness of the body which lacks the fresh turgid appearance of a healthy specimen. It is considered that these infestations may materially affect the mortality rate of the males. It is noteworthy that in the Outer Grounds the larger percentage of females in the population is accompanied by a very heavy parasitic infection of the males in that area. In the Rame-Eddystone Grounds, on the other hand, where the sex ratio is nearer equality, the gonad parasite has not been recorded. In the Plymouth Sound population the percentage of females was slightly higher than in the Rame-Eddystone Grounds, and this may similarly be associated with the presence of the parasite in the former population, causing greater mortality of males, thus giving an increase in the percentage of females. The parasite has not been recorded in the E 1 area, but the few samples from that locality were all taken too late in the season for the presence of the parasite to be expected. Nor has the parasite been recorded in the Rame Mud population of small starfishes.

The parasite only occurs in the testes during January–April with an occasional occurrence in May. It is reasonable to suppose that, if the parasite *Orchitophrya* has an adverse effect on the mortality rate of the male starfishes, the losses due to it should occur around that time, i.e. spring and early summer. It is, in fact, considered that the rise in the percentage of females which occurs at that time is due to a differential mortality caused partly by the weakening effects of the parasite *Orchitophrya* which only attacks medium- and large starfishes with well developed gonads, and partly by a general weaker constitution and shorter life span of the males. These factors would tend to reduce the percentage of males in a population of large and medium sized starfishes. This decline in the numbers of males in a population would be cumulative if it were not made good by the growth of smaller males, which reach a size large enough to be taken by the trawl and are thus included in the population samples, or by the gradual immigration from poorly fed populations of small starfishes such as that on the Rame Mud which have an excess of males.

SUMMARY

The sex ratio of *Asterias rubens* in five populations near Plymouth has been obtained from observations on over 4000 specimens. The overall percentage of females in samples of large and medium-sized starfishes was 51·3 in the Rame-Eddystone Grounds, 55·1 in the Outer Grounds and 70·3 in the E 1 area; in the isolated Plymouth Sound population the percentage was 53·6. In the Rame Mud population of small starfishes (taken only by Agassiz trawl) the percentage of females was 45·6.

On the Outer Grounds and Rame-Eddystone Grounds the percentage of females was found to vary according to the time of year, being highest in March–June and lowest in January–February.

In a general discussion of these results it is considered that they are probably not related to any change of sex in the individuals, as no trace of hermaphroditism has been found in many thousands of gonads examined. It is also

unlikely that there is any degree of differential sexual swarming. It is, however, considered possible that the rise in the percentage of females is due to a greater mortality rate in the males. There is indirect evidence that males are in general less resistant to injury than females, and it is known that they are liable to gonad parasitization by *Orchitophrya*. It is suggested that a greater male mortality rate among medium and large starfishes may be due to both these factors, and that the resulting deficiency in males is made good by recruitment of small starfishes among which males preponderate, either from the same grounds or from neighbouring grounds.

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THE ANNUAL GROWTH AND REPRODUCTIVE CYCLE IN FOUR ASCIDIANS

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(Text-figs. 1–11)

This study was undertaken to increase our very incomplete knowledge of the annual cycle in ascidians.

Berrill (1950) states that 'the age of ascidians, with one exception, is practically impossible to estimate, unless a certain inhabited area is followed closely through seasons and years'. In the present work the method has been to follow certain aspects of the ascidian population of a chosen area for a period of nearly two years. The area was the Old Dock in Ardrossan Harbour, Ayrshire, and the ascidian population contained four species: *Diplosoma listerianum* (Milne Edwards), *Ciona intestinalis* (Linnaeus), *Ascidiella aspersa* (Müller) and *Botryllus schlosseri* (Pallas). *Diplosoma* belongs to the order Enterogona, suborder Aplousobranchiata; *Ciona* and *Ascidiella* to the order Enterogona, suborder Phlebobranchiata; and *Botryllus* to the order Pleurogona, suborder Stolidobranchiata. Thus each of the three main suborders is represented, and the four ascidians studied are amongst the commonest British species.

There are three main aspects of the annual cycle: (i) growth, (ii) number and life-span of generations in the year, (iii) sexual reproduction.

Growth. In the simple ascidians *Ciona* and *Ascidiella* increase in length of the body suffices as a measure of growth. In the compound forms *Diplosoma* and *Botryllus*, however, the case is complicated by the existence of budding. This may involve rapid and almost continual production of new individuals which replace, or live along with, old ones in the colony. It is therefore difficult to find a significant measure of growth in these compound forms. The most convenient measurement, however, is area, since during growth the colonies of both *Diplosoma* and *Botryllus* increase in surface area but scarcely at all in thickness.

Number and life-span of generations in the year. The appearance of a new generation and the disappearance of an old one can be detected by inspection of the samples. It is difficult to see the small newly settled larvae, however, and the first evidence of a new generation is usually the presence of small individuals or colonies which have been established for a short but unknown time.

Sexual reproduction. Faunistic lists and papers sometimes give notes on the breeding season of various species, but these are usually generalized conclusions based on observations made over a number of years. Also they fail to define the term 'breeding season', which might include a variety of conditions and times from the onset of gonadial development to the settlement of larvae. The period when an ascidian carries eggs and sperm is often considerably longer than the period of their liberation and development in nature. This latter period depends on internal and external limitations on spawning and successful development.

In the present study the progress of breeding was followed by noting the periods when animals carried eggs and sperm, developing eggs, or larvae, and by finding the period during which larvae had settled.

The quantity of eggs and sperm in the ducts can be readily seen in *Ciona* and *Ascidiella*. *Diplosoma* and *Botryllus*, however, do not have long genital ducts in which eggs and sperm are stored, and in these animals the reproductive condition was assessed by the state of development of gonad, and by the presence or absence of developing eggs and larvae in the zooids or the colony.

THE AREA STUDIED

The Old Dock, Ardrossan, is a semi-tidal sea-water dock, the water-level in which is controlled by sea gates. The amount of interchange of water between the dock and the sea is uncertain and variable, and depends partly on the traffic of ships through the gates. The predicted depth at H.W.O.S.T. is 19 ft. and the area of the dock is 4·23 acres. The walls, which are of stone, are vertical and form the substratum to which *Ciona* and *Ascidiella* are attached. *Diplosoma* and *Botryllus* are generally fixed to the test of the simple ascidians.

METHODS

Samples were collected by scraping the walls of the dock at intervals throughout 1950 and 1951.

The size of samples varied with the availability of material, which depended partly on the time of year and partly on the water-level in the dock. The number of specimens measured (N) is shown on histograms of size distribution where percentages are used. The animals were narcotized with menthol, fixed in the expanded state in strong formalin and stored in weak formalin. Measurements of length in *Ciona* and *Ascidiella* were made to the nearest 1 mm. The colonies of *Diplosoma* and *Botryllus* were carefully removed from the substratum (generally the test of *Ciona* or *Ascidiella*), placed flat on the glass negative-carrier of a photographic enlarger, covered with a glass slide, and inserted in the enlarger. The outline of the projected image was traced on squared paper and the area of the colony calculated from the outline tracing.

Specimens of *Ciona* and *Ascidiella* were dissected and the genital ducts examined for eggs and sperm. A number of zooids were removed from each colony of *Botryllus* and examined for the presence of gonad, developing eggs and larvae. In the colonies of *Diplosoma* zooids were examined for eggs, and the common test for developing eggs and larvae. In each sample of *Botryllus* and *Diplosoma* about twenty colonies were thus examined. Pieces of several colonies of the compound forms, in each sample taken during 1950, were sectioned to confirm the results of dissection.

Ascidiella aspersa (Müller)

Growth, number of generations in the year, and life-span.

The length of the specimens was taken as a measure of their size. In Fig. 1 a series of histograms shows the distribution by size of *A. aspersa* in the samples taken in 1950 (left) and 1951 (right).

The sample of 24 January 1950 showed two populations, one whose body length had the mode about 25 mm., and the other with the mode about 50 mm. The population of small animals represented rather more than 80% of the sample and consisted of animals that settled as larvae in the previous summer (1949). The remaining population, of large specimens, was rather less than 20% of the sample and represented animals that settled as larvae in the summer of 1948.

Between January and April there appears to have been some growth of the smaller animals of the 1949 group, but less amongst the larger ones. During this period the percentage of the total sample represented by the 1948 group gradually decreased until in April it accounted for only about 2% of the total sample. Thus, although a large part of the 1948 group had perhaps died before the first sample was taken in 1950, it was in the months from January to April that this group virtually disappeared.

Growth for the 1950 season became important between 21 April and 24 May and continued at a high rate until about the middle of June. From then until the middle of July growth was considerably slower, at least amongst the larger individuals.

The sample of 14 July gave the first evidence of the new generation, and from this time until about the end of September growth of animals of this 1950 group continued. During the same period there was no appreciable growth in specimens of the 1949 group, and after the end of September there appeared to be no further growth in either the 1949 or the 1950 group.

On 27 November about 20% of the sample belonged to the 1950 group and about 80% to the 1949 group.

It is evident that in 1950 only one new generation of *A. aspersa* was produced, and that this was established mainly in July but possibly also somewhat earlier and later.

The samples of 11 January and 13 April 1951 are too small to be very useful but tend to confirm the results of 1950, that at this early period of the year

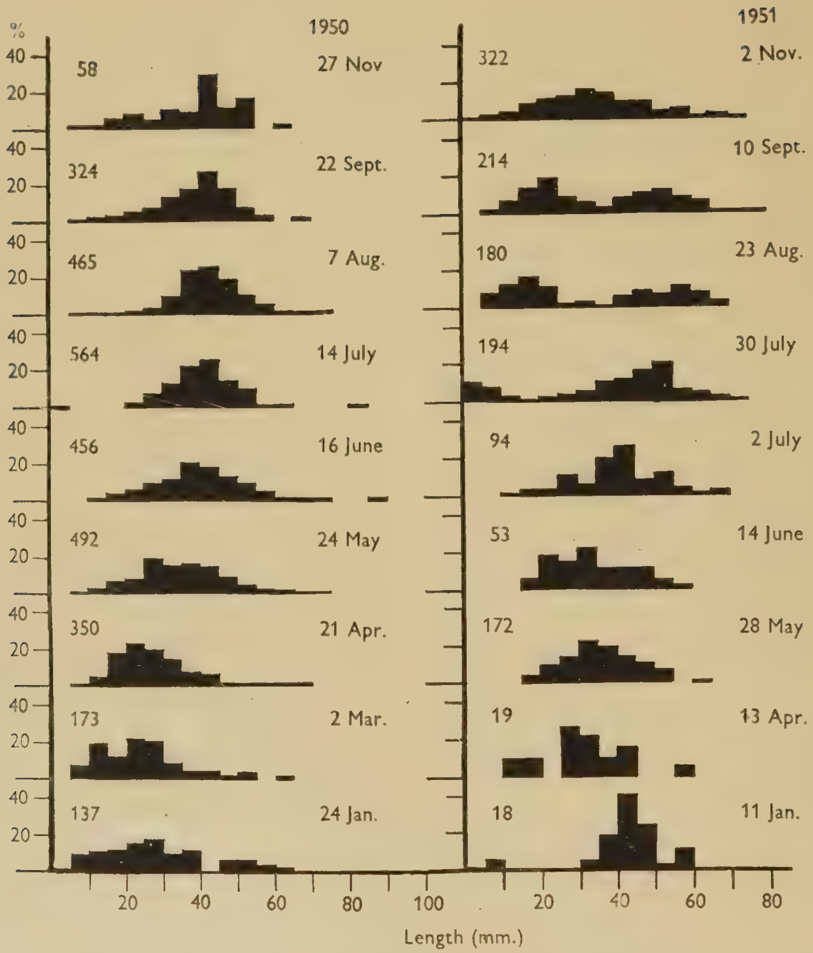

Fig. 1. Size-frequency histograms (in percentages) of *Ascidiella aspersa* in 1950 and 1951. The figures on the left of each histogram denote the number of specimens in the sample.

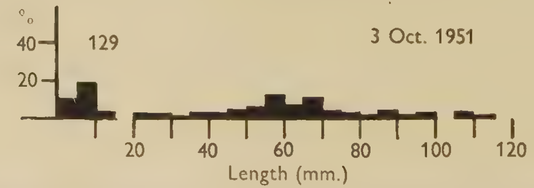

Fig. 2. Size-frequency histogram of *A. aspersa* on 3 October 1951, from Loch Sween, Argyll; as in Fig. 1.

the population represents two year-groups. The animals of these two groups had settled as larvae in the summers of 1949 and 1950 respectively. The samples also suggest that many of the larger individuals (i.e. of the 1949 group) died between 11 January and 13 April.

The figures for the rest of 1951 follow the same general pattern of growth and settlement that was found in 1950. In 1951, however, no growth was apparent until after 14 June, whereas in 1950 the animals had grown considerably before 24 May. On 2 July no newly settled individuals were found. They were present on 30 July, however, but in the sample of 23 August and in all later samples none was found. The period of larval settlement in 1951 therefore started between 2 July and 30 July and finished between 30 July and 23 August.

Animals of the new, 1951, group continued to grow from the end of July until some time between 10 September and 2 November, but during this period the animals of the old, 1950, group made no measurable growth. Only one new generation was established in 1951.

A single sample of *A. aspersa* was taken from Loch Sween, Argyll, on 3 October 1951 to provide a comparison with the autumn samples from Ardrossan. Fig. 2 shows the size distribution of the specimens. It appears that here also the population represents two clearly separate age-groups, the smaller animals, up to about 15 mm. long, belonging to the 1951 settlement and the larger ones belonging to the 1950 settlement. The size distribution of this sample suggests that the general pattern of growth and reproduction may be similar over much of the west coast of Scotland. The main differences in the sample from Loch Sween, as compared with autumn samples from Ardrossan, are: (i) the smaller size of the individuals of the 1951 group, and (ii) the larger size of those of the 1950 group.

These features may result from a later breeding season and more favourable conditions for growth in Loch Sween.

It may be deduced, from the samples of these two years, that the life-span is of the order of 18 months, extending approximately from the middle of one summer until the winter of the following year. *A. aspersa* in this area is essentially an annual, a conclusion which Hŭus (1937) also reached for this species on the Norwegian coast.

Sexual maturity

Fig. 3 shows, for each of the size-groups, the percentage of sexually mature animals in the samples of 1950. The upper half (*S.*) refers to sperm, and the lower half (*E.*) to eggs, in the genital ducts. Sexual maturity is here taken to mean the presence of sperm or eggs in the genital ducts, irrespective of the quantity. The figures show two features:

(1) *A. aspersa* is hermaphrodite, but is slightly protandrous, as the sperm duct starts to fill before the oviduct. The 20–25 mm. group shows this

protandrous tendency most clearly. This finding contradicts Herdman's (1899) general statement that *Ascidia* is protogynous.

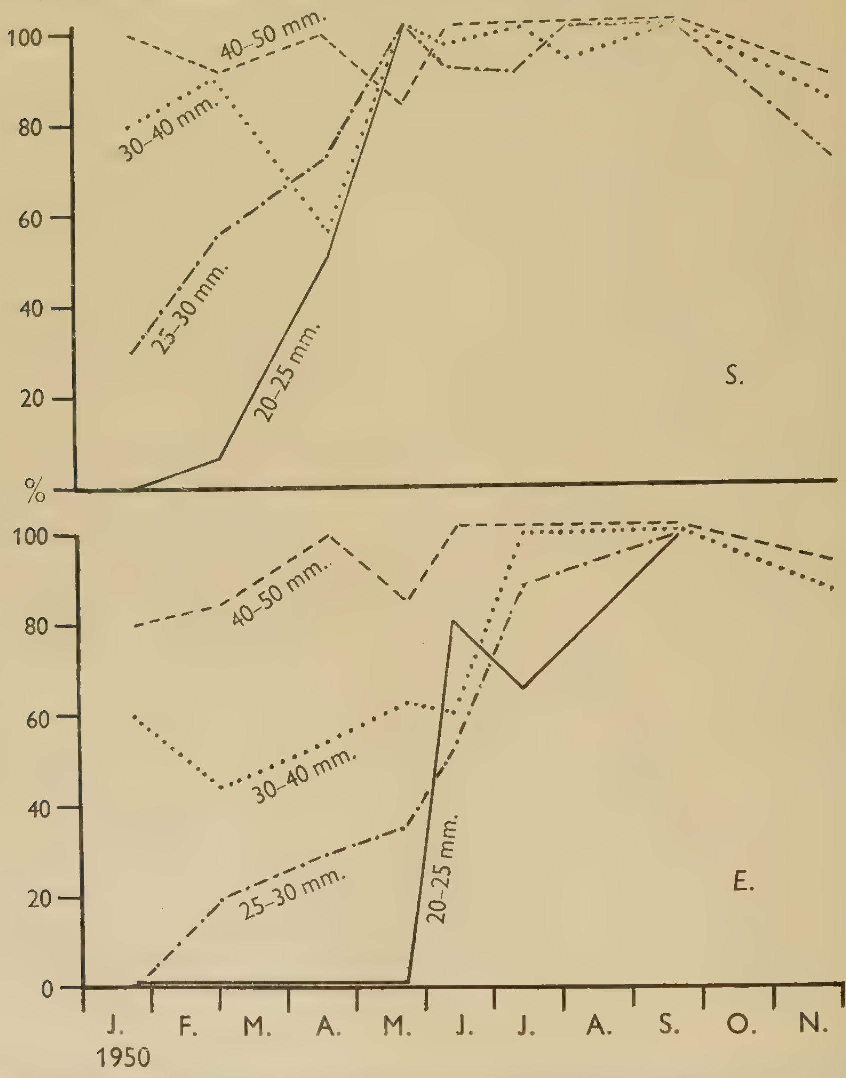

Fig. 3. Relations between the time of year and the percentage of specimens of *A. aspera* with sperm (*S.*) and eggs (*E.*) in the ducts, for each of the size groups 20–25, 25–30, 30–40 and 40–50 mm., during 1950.

(2) Sexual maturity depends primarily on the size of the animal. When an individual reaches a certain critical size sperm begins to appear in the sperm duct and at a slightly larger size eggs pass into the oviduct. The critical size, however, changes during the year. Thus practically all individuals over

40 mm. in length are mature as male and female throughout the year. In the 30–40 mm. group over 80% are mature as males during the whole year, but from January until June only about 40–60% of this group are mature as females. In the 25–30 mm. group there is a steady rise in the proportion with sperm, from January to May, when all have become mature; there is a similar but more gradual rise in the proportion with eggs. The 20–25 mm. group shows the sharpest rise in the percentage mature, both as male and as female, between January and June. In June sperm generally starts to fill the sperm duct when the animal is slightly under 25 mm. long, and eggs pass into the oviduct when the animal is rather less than 30 mm. long. This difference of about 5 mm. in the critical body length for male and female maturity is supported by observations made on a sample of *A. aspersa* taken on 20 August 1948 from a tufnol plate suspended in the waters of Loch Sween, Argyll. Fig. 4 shows, for this sample, the relation between the body length and the presence or absence of sperm and eggs in the ducts. Incidentally, the animals in Loch Sween became mature at a smaller body size than did those in any of the samples from Ardrossan. This may have resulted from environmental differences like those which appear to have influenced the Ardrossan population during the course of the season.

The time of settlement, growth rate, critical body length and life-span were such that a sample taken at Ardrossan at any time of year contained a large proportion of mature individuals. In the sample of 27 November 1950, for example, most of the 1949 group carried eggs and sperm, and the larger members of the 1950 group also did so. Of this population, however, the 1949 group died before spawning again, in 1951. Its place was taken by the 1950 group, which spawned in the summer of 1951. In the area studied, therefore, it appears that a given year group of *A. aspersa* has only one spawning season, and that is in the year after it settled as larvae.

Ciona intestinalis (Linnaeus)

Growth, number of generations in the year, and life-span

Ciona is more difficult to measure than *A. aspersa* because it is more contractile and because its body form varies more according to its contact with the substratum. It was therefore essential to narcotize the animals thoroughly before fixing and measuring them. Another difficulty was the limited supply of animals necessitating small samples.

Fig. 5 is a series of histograms showing the distribution by size of *Ciona* taken at intervals throughout 1950 and part of 1951. The samples of January, March and April 1950 showed two populations, but these were not clearly distinguishable. The numerically larger population had a small body length, and most specimens were between 10 and 30 mm. long. These animals had settled as larvae in 1949. The other population had a body length ranging

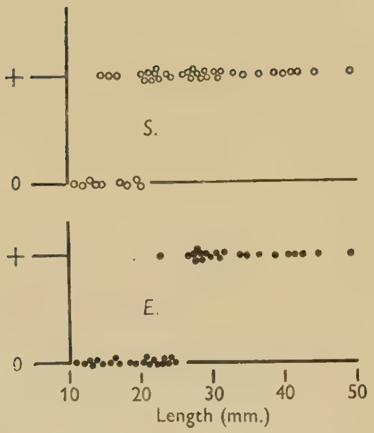

Fig. 4.

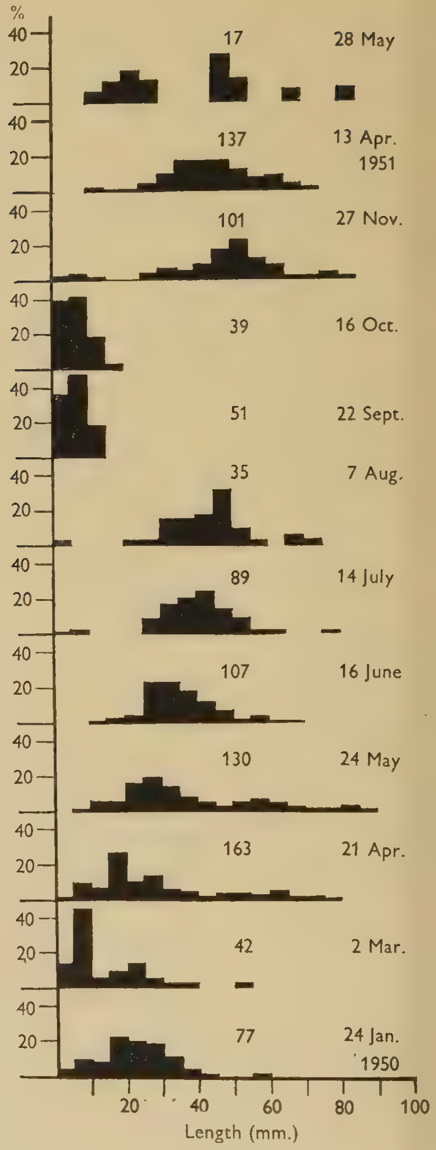

Fig. 5.

Fig. 4. Relation between the body length and the presence or absence of sperm and eggs in the ducts of *A. aspersa*, in a sample from Loch Sween, Argyll, taken on 20 August 1948. *S.* sperm in duct; *E.* eggs in duct.

Fig. 5. Size-frequency histograms (in percentages) of *Ciona intestinalis* in 1950 and early 1951. The figures denote number of specimens in the sample.

approximately from 50 to 70 mm. These animals had settled as larvae in 1948. The 1949 group tended to be bimodal, suggesting that during the previous year settlement may have been more intense at two periods separated by an interval when fewer larvae settled. Little or no growth was evident between January and 21 April, but during May, June and July the animals of the 1949 group grew considerably. By the end of May the bimodal tendency could no longer be detected in the 1949 group, the smaller individuals having grown faster than the larger ones. The animals of the 1948 group made little, if any, growth between May and July, as most of them had already reached their maximum size. There are, unfortunately, gaps in the records of the 1949 group during September and October, as continued sampling had reduced the stock. It was not until November that numbers of this population were found in a different part of the dock. These gaps in the records, however, are not serious, since it is obvious from the histograms for August and November that we are dealing with the same year-group. Between August and November there appears to have been little growth within the 1949 group.

It is more difficult to assess the proportion of the 1948 and 1949 groups in each sample of *Ciona* than in the samples of *Ascidiella*, and to say when the 1948 group died out. Most of the 1948 group had apparently disappeared before the first sample was taken in 1950, but a few were certainly still present in the sample of 24 May.

The first evidence of the 1950 settlement was in the sample of 14 July, in which a few young specimens were found. As one of these was already 7 mm. in length, settlement of larvae must have started a few weeks before this sample was taken. It was difficult to find numbers of the 1950 group sufficient to give a clear idea of their growth rate. This may have been due partly to depleted breeding stock and partly to an unsuccessful breeding season. The similarity between the histograms for 22 September and 16 October suggests that growth of the members of the 1950 group did not continue after the end of September.

Only one generation of *Ciona* was produced in 1950 in the area studied. Settlement of larvae appears to have started in July and continued through August and probably into September, as at the end of this month specimens of only 2 mm. length were still being found. The presence of individuals in the 0–5 mm. group during October and November does not necessarily indicate further settlement. As growth had already stopped for the year individuals which settled in September could still be in the 0–5 mm. group throughout the winter. This suggestion is supported by the presence of specimens only 3 and 4 mm. long in the samples of January and March 1950.

In 1951 samples were obtained up to 28 May after which the population was apparently so depleted that only occasional specimens were taken. These few samples, however, tend to confirm two features of the 1950 sampling: (i) growth of the animals of the recent year-group (in this case the 1950 group) started before the end of May; and (ii) most of the remaining animals of the previous year-group (in this case the 1949 group) died before the end of May.

The general pattern of growth, reproduction and replacement of generations in *Ciona* was very like that found in *Ascidiella*. Individuals settled in the summer, grew until autumn, resumed growth in the following spring, spawned in the summer of that year, and died in the following winter. They are therefore essentially annuals, although they may be 18 months old or somewhat more when they die.

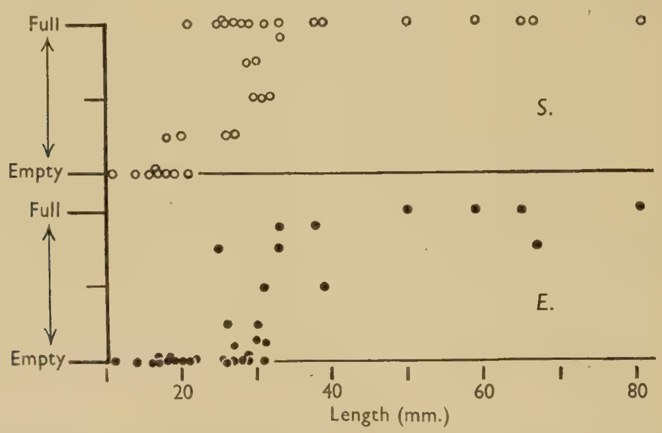

Fig. 6. Relation between the body length and the quantity of sperm (*S.*) and eggs (*E.*) in the ducts of *C. intestinalis* in the sample taken on 24 May 1950.

Orton (1914, 1920) found that in the Plymouth area *Ciona* breeds from April to November, producing two or three generations in the year. Hŭus (1937) states that on the Norwegian coast *Ciona* is sexually mature within its first year of life and dies off after breeding, having a life of about one year. In Naples, Lo Bianco (1909) records *Ciona* as breeding throughout the year. Rünnstrom (1936) found that *Ciona* has three physiological races which breed within different temperature ranges. On the west coast of Norway he found *Ciona* to spawn from May to August, after which the old generation dies off. It is replaced by the new one which becomes sexually mature by the end of August but does not, apparently, produce a further generation: eggs and sperm may be shed from October to December but fail to develop.

The population of *Ciona* studied in Ardrossan Old Dock resembles the animals of the Norwegian west coast in breeding behaviour and the annual cycle of generations, although differing somewhat in the timing of events and speed of processes.

Sexual maturity

Fig. 6 shows the relation between the length of the body and the quantity of eggs and sperm in the genital ducts of a number of specimens of *Ciona* from the sample of 24 May 1950. The remaining samples were not examined in detail, but the same general relations were found.

Ciona, like *Ascidiella*, is a slightly protandrous hermaphrodite. Sperm starts to enter the sperm duct at a critical body length and eggs appear in the oviduct later, when the body length is somewhat greater. Fig. 6 suggests that the critical body length for the appearance of sperm in the duct is about 25 mm. and of eggs in the oviduct about 30 mm., in the sample examined.

Diplosoma listerianum (Milne Edwards)

Growth

The area of the colonies was measured as described on p. 42. In Fig. 7 a series of histograms shows the distribution by area of the colonies taken at intervals throughout 1950 and 1951.

All colonies of the sample of January 1950 originated from larvae that settled in 1949. Most of the colonies of this sample were less than 50 mm.^{2} in area and the largest was 170 mm.^{2}. Growth in 1950 started between 2 March and 21 April and continued until about the end of May. Maximum size appears to have been reached by 16 June, when a number of colonies were between 1000 and 1500 mm.^{2} in area.

In the sample of 14 July a few colonies showed degeneration of the zooids, but it was in the sample of 7 August that this process first became widespread. Food was not seen in the gut of zooids in August, and this is taken as another sign of lowered vitality. By 22 September 1950 most of the 1949 generation of colonies had died and many of those remaining were degenerate.

New colonies of the 1950 generation were first observed in the sample of 14 July but were few. The new colonies were more common on 7 August. On 22 September most of the sample, and on 27 November all of it, consisted of the new, 1950, generation.

Appreciable growth of the newly established colonies took place before 22 September.

The pattern of growth and replacement of generations in 1951 was in general similar to that observed in 1950. In 1951, however, spring growth up to 28 May was much less than spring growth during a corresponding period of 1950 (up to 24 May). A similar feature was observed in the growth of *Botryllus* (see p. 58). In the autumn of 1951 colonies of the new generation continued to grow after 10 September, as shown in Fig. 7, bottom right.

Sexual reproduction

Asexual reproduction by budding leads to an increase in the size of the colony and not to the founding of new colonies, which is the function of

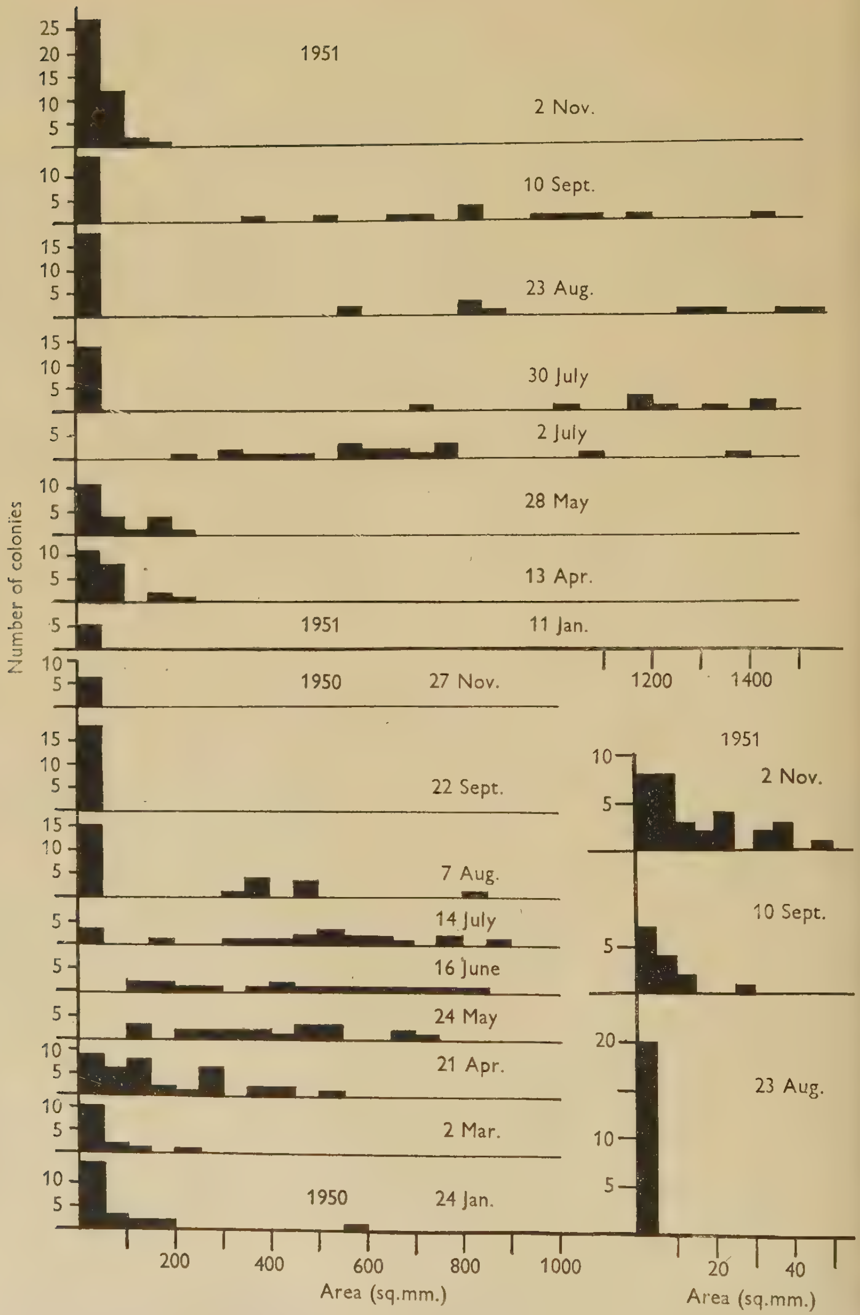

Fig. 7. Size-frequency histograms (by area) of *Diplosoma listerianum* in 1950 and 1951. The smallest specimens for three successive dates are further analysed in the inset at right bottom.

sexual reproduction. During sexual reproduction budding was reduced but it continued long after the onset of gonadial development and egg production. It only ceased, presumably, when there was serious competition for raw materials between the sexual and the asexual processes. Huxley (1921) and Berrill (1935) have drawn attention to this competition in colonial ascidians.

The ovary (Fig. 8, *Ov.*) of *Diplosoma* lies in the lower part of the abdomen, and shows only a few large eggs at any time. As the season advances the eggs enlarge and pass singly from the abdomen down into the basal layer of test. Lahille (1890) states that the egg is fertilized only after breaking through the body wall and coming to lie in the common test, but Berrill (1950) maintains that it is fertilized before leaving the abdomen. Development to the larval stage certainly takes place within the common test.

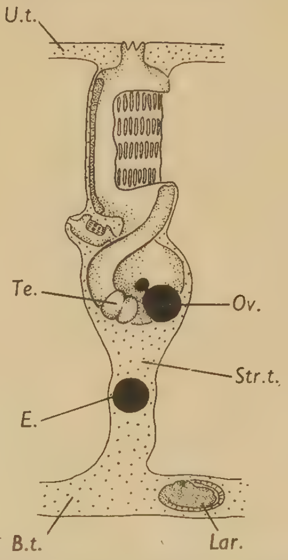

Fig. 8. Zooid of *D. listerianum* within the common test of the colony. *B.t.* basal layer of common test; *E.* egg; *Lar.* larva; *Ov.* ovary; *Str. t.* strand of common test securing zooid to basal layer; *Te.* testis; *U.t.* upper layer of common test.

Fig. 9 summarizes the conditions found in the samples of *Diplosoma* throughout 1950, and records the percentage of colonies with: (A) neither eggs nor larvae in zooids or test; (B) eggs in zooids but not in test; (C) eggs in zooids and eggs or larvae in test; (D) eggs or larvae in test but no eggs in zooids; (E) larvae.

The graphs indicate the progress of breeding activity.

January to March was a period of sexual inactivity, during which colonies of the 1949 generation had eggs neither in zooids nor in test. Some colonies, however, showed eggs or larvae, or the degenerate remains of these, in zooids or test. These eggs and larvae appear to be residual products which failed to develop completely and escape during the previous year. They may be regarded as abnormal, as they were probably held within the incompletely disintegrated basal layer of colonies of the 1948 generation. This condition was more common in 1951 (see p. 55).

Between the March and April samples there had been an increase in the proportion of colonies with eggs in the ovaries, and this period marks the onset of sexual activity for the year. During May there was a sharp rise in the percentage of colonies with eggs in the common test as well as in the zooids, ndicating a large-scale passage of eggs from the zooids down into the common test. It was on 24 May that the first developing embryos were seen. All colonies examined in the middle of June had eggs in the zooids and also eggs or larvae in the test. Egg production started to diminish in July, as indicated by a slight drop in the percentage of colonies with eggs in zooids as well as test. Between mid-July and early August the ovary became inactive and the production of eggs ceased. In the July and August samples all colonies examined had larvae, and some had developing eggs, in the test.

The first signs of exhaustion resulting from breeding activity were seen in July, when a few colonies were found with degenerating zooids; in August this condition was common.

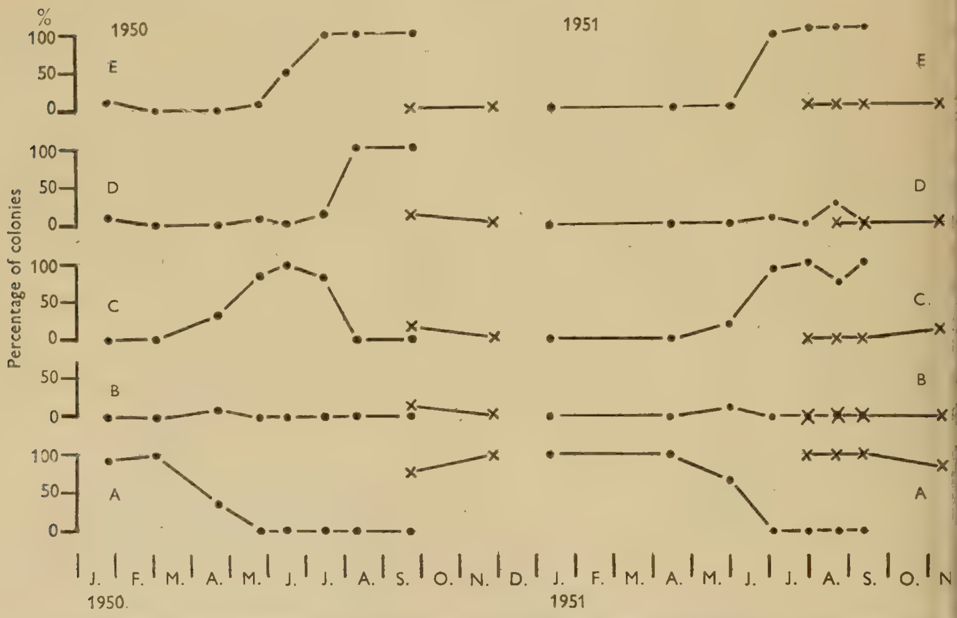

Fig. 9. The breeding activity of *D. listerianum* in 1950 (left) and 1951 (right). A, colonies with no eggs and no larvae in zooids or test; B, colonies with eggs in zooids but not in test; C, colonies with eggs in zooids and eggs or larvae in test; D, colonies with eggs or larvae in test but no eggs in zooids; E, colonies with larvae.

Larval settlement was indicated by the presence of young colonies in the samples of 14 July, 7 August and 22 September. The settling period was not determined more precisely and may not have extended into the second half of September. It is perhaps significant that the settling period coincided with the period during which the old colonies disappeared, and it may be that the larvae are liberated only with the disintegration of the breeding colonies. Berrill (1950), on the other hand, states that the active tadpoles escape from the test matrix to the common cloacal cavities of the colony and thence to the exterior. Many of the Ardrossan colonies examined, however, showed areas of degenerating zooids below which larvae were still retained in the common test. The existence of many composite colonies (see below) in November 1951 also indicates prolonged retention of larvae in the test, even after the disappearance of the parent zooids.

The young colonies grew and, as the September sample showed, some of the zooids produced eggs. Developing larvae were also found in a few young colonies in September. That some of these young colonies may have given rise to a second generation is suggested by the presence of a few two-zooid colonies in late November. It is possible, however, that these small colonies in November came from late larvae of the old colonies.

A study of the breeding activities of *Diplosoma* during 1951 confirmed the general picture gained in 1950, as seen from the right-hand part of Fig. 9. It appears that the onset of breeding was later in 1951 than in 1950. This difference is shown in the later disappearance of the non-breeding condition (A), the later appearance of eggs in the zooids (B), the later rise in the percentage of colonies with eggs in both zooids and test (C), and the later transition of colonies to the final breeding stage (with eggs or larvae in the test only, D). By comparing the dates in 1950 and 1951 on which the same percentage of the samples was in a particular reproductive state, we can estimate the lateness of the 1951 season. The lateness was of the order of 6 weeks.

One other feature of the 1951 graphs deserves comment: this is the much smaller percentage of colonies during August with eggs or larvae in the test only, compared with the 1950 figures. The lower percentage in 1951 was due to the later degeneration of zooids, because as long as the zooids remain healthy some at least continue to produce eggs.

The delayed decay of the old colonies in the autumn of 1951 gave rise to another interesting condition: this was the formation of composite colonies. A composite colony consists of the basal layer of test of an old colony with its larvae still enclosed, and zooids of the new generation derived from these larvae which have metamorphosed *in situ*. No zooids of the old generation remain.

Later breeding in 1951 may have resulted from later growth, which was observed to occur, but more probably some environmental factor, such as lower water temperature, retarded both activities.

Number of generations

In the area studied *Diplosoma* produced, both in 1950 and 1951, one principal generation and possibly a minor one in 1950 late in the season. Orton (1914) states that, in the Plymouth area, the species passes through 'at least two crops in a year' and three or more in favourable seasons. Once he got larvae from a colony aged not more than 3 weeks and 5 days.

The rate of development of the colonies and the time at which larvae are liberated are the factors determining the number of generations per year.

These factors are greatly influenced by local and seasonal conditions. Berrill's (1950) statement that 'breeding occurs throughout the year' is too general, although no doubt true in specially favourable conditions.

Botryllus schlosseri (Pallas)

Growth

The colonies of *Botryllus* were removed from the substratum and their area measured by the method described on p. 42. Fig. 10 gives a series of histograms showing the distribution by area of the colonies taken at intervals throughout 1950 and 1951.

In January 1950 the sample of *Botryllus* represented a single year-group, consisting of colonies that had been established in 1949. Many colonies were under 25 mm.^{2} in area and had only one system of zooids. The few large colonies in this sample were between 200 and 300 mm.^{2} in area and these had ten to twelve systems of zooids. The onset of growth for the 1950 season occurred between 2 March and 21 April. In the April sample most specimens lay within the 25–30 mm.^{2} group and these colonies had two or three systems of zooids. Large colonies between 500 and 750 mm.^{2} had fifteen to twenty systems. Growth continued during May and by 16 June maximum size had, in general, been attained. Only two colonies in the June sample had as few as six systems of zooids and the majority had fifteen to twenty or more systems. One or two of the large colonies showed some degeneration in June, the first to be observed in 1950.

The sample of 14 July showed two important features: (1) the first new colonies resulting from larval settlement, and (2) considerable degeneration in the large colonies.

The new generation was represented by two small colonies, each of a few zooids arranged in one system.

By 7 August the change of generations was almost complete, only one colony of forty-three examined belonging certainly to the 1949 generation. The remainder of this sample consisted of small colonies, mostly of one system, but a few of two, three, or four systems. Two single zooids were found, recently metamorphosed from settled larvae. On 22 September no colonies of the 1949 generation were found.

A more detailed comparison of the samples of 7 August, 22 September, and 27 November 1950 shows that the young colonies grew during late summer and autumn (Fig. 10, inset). Table 1 suggests that this growth took place without an increase in the number of systems per colony.

It can be seen by inspection of the histograms for 1951 that they agree fairly well with those for 1950. In the sample of January 1951 all colonies belonged to the 1950 generation. Growth had begun by 13 April and continued until June. Early in July degeneration of colonies had started but was most noticeable at the end of that month. The first new colonies, of the 1951

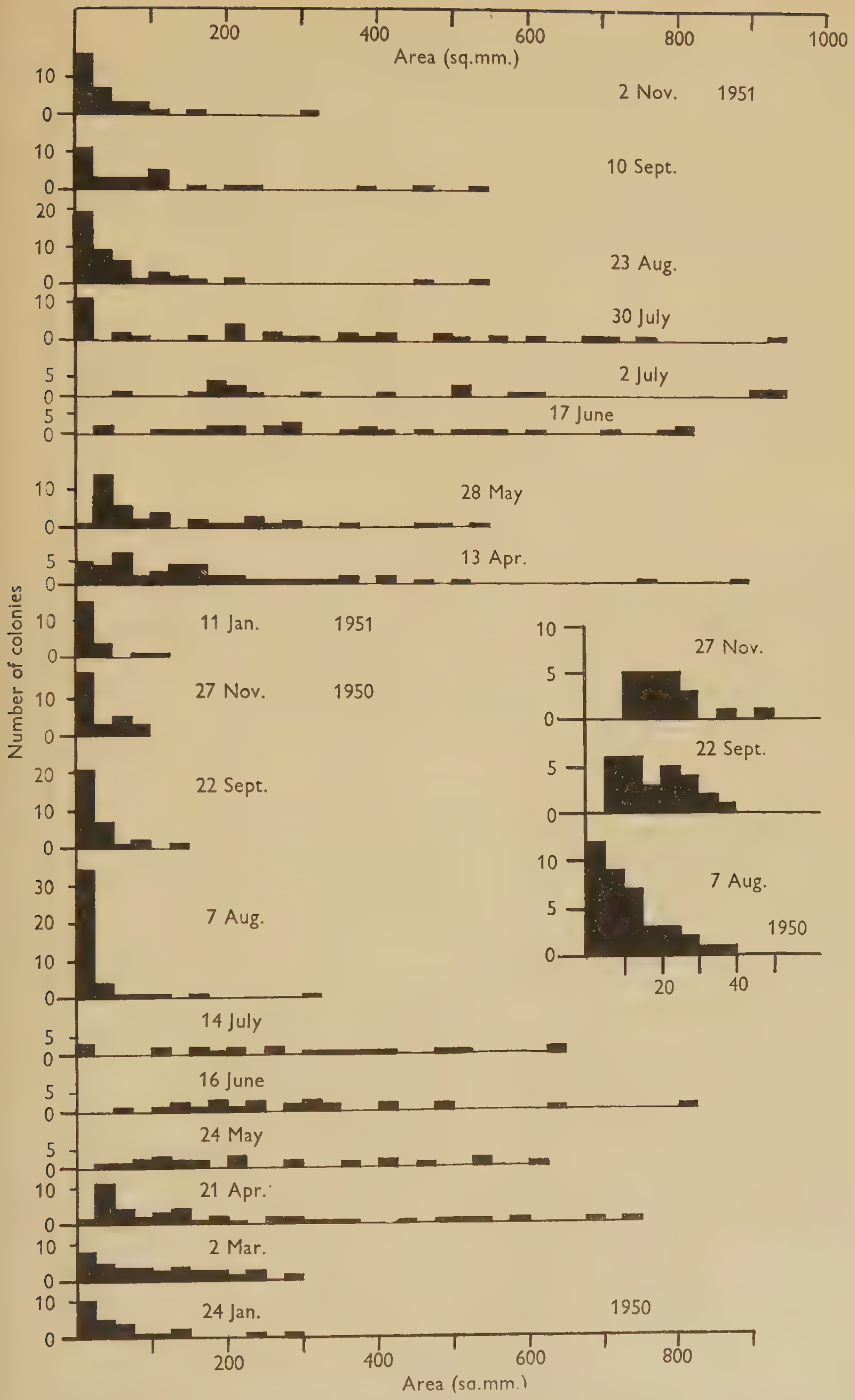

Fig. 10. Size-frequency histograms (by area) of *Botryllus schlosseri* in 1950 and 1951. The smallest specimens for three successive dates in 1950 are further analysed in the inset on the right.

generation, were seen at the end of July, and by 23 August most of the sample consisted of this new generation.

Although the general pattern of growth was similar in the two years, the onset of spring growth was later in 1951 than in 1950. This can be seen by comparing the histograms for approximately corresponding dates in the two years, viz. 21 April 1950 and 13 April 1951; 24 May 1950 and 28 May 1951. A similar retardation of growth was noticed in *Diplosoma*.

TABLE I

No. of systems per colony	No. of colonies			
	7. viii. 50	22. ix. 50	27. xi. 50	11. i. 51
A few zooids	2	0	0	0
1	24	20	15	16
2	9	5	3	1
3	4	2	4	2
4	1	2	5	0

Number of generations

The population of *Botryllus* passed through one generation in each of the years studied, and it seems unlikely that even the earliest new colonies were able to produce many larvae that settled to form a second new generation. Orton's (1914) findings that *Botryllus*, like *Diplosoma*, has at least two crops in the year applies to the Plymouth area.

Sexual reproduction

Berrill (1941, 1947, 1950) has described the process of bud formation and gonad development in *Botryllus* and *Botrylloides*. He has shown that the sex of the gonad and the state of development that it attains depend on the size of the bud bearing the gonad. Berrill (1935) also found that sexual and asexual reproduction take place simultaneously, but that budding may be relatively subdued during sexual reproduction.

The samples of *Botryllus* were examined, and Fig. 11 records the percentage of colonies in each sample during 1950 and 1951 with: (A) neither eggs nor larvae, (B) gonads, (C) large or developing eggs, (D) larvae.

Gonads were visible in a low percentage of colonies in January and March, and sections showed small developing ovaries and testes in some zooids and buds. No larvae were present in these months and only a few large eggs were found in one colony in March. Gonadial development was rapid during March and early April, but still there were only a few large eggs, and no larvae. The percentage of colonies with visible gonads was highest in May and June, dropped sharply to the level of July, and by late September had returned to the winter level. The percentage of colonies whose zooids had large or developing eggs followed a rather similar curve, but reached a high value for a shorter period.

Larvae were first found in the sample of 24 May, when some colonies had fully developed larvae and others had tailed but still incomplete larvae. The percentage of colonies bearing larvae continued to rise in June and reached a maximum in July, after which there was a rapid decrease. A few larvae were still found until the end of November. The first new colonies of the year appeared in the sample of 14 July and their number increased in August, but there was no evidence of very recent settlement in the sample of 22 September.

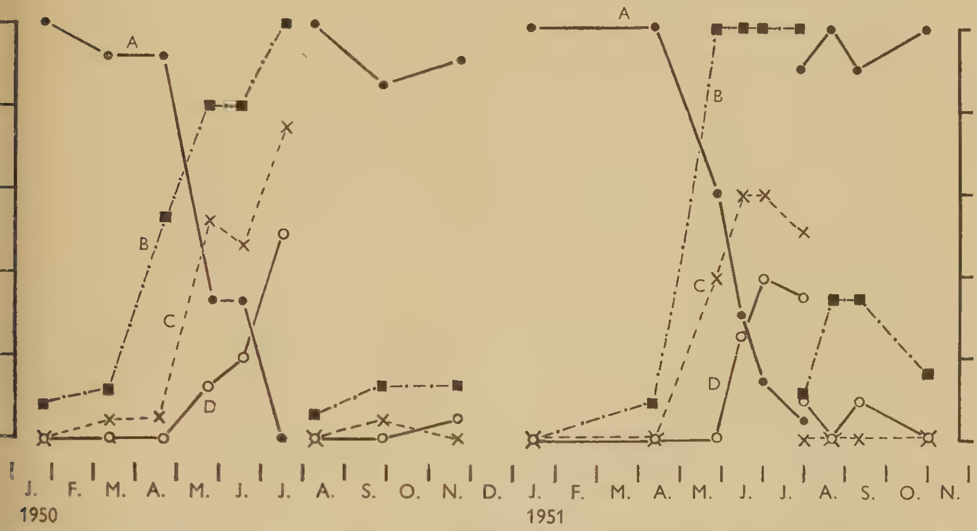

Fig. 11. The breeding activity of *B. schlosseri* in 1950 (left) and 1951 (right). A, colonies with no developing eggs and no larvae; B, colonies with visible gonad; C, colonies with developing eggs; D, colonies with larvae.

In 1951 the cycle of breeding in *Botryllus* was similar to that in 1950, but was later in most of its stages. This appears from a comparison of the graphs, for the two years, of the percentage of colonies with (1) gonad, (2) developing eggs, and (3) larvae. The period of larval settlement, however, was rather similar, starting some time between 2 July and 30 July, and extending certainly until the end of August and perhaps into September.

In both years the proportion of *Botryllus* colonies with larvae reached, at its maximum, only about 30–40% of the total examined. This is in marked contrast to the condition in *Diplosoma*, where 100% of the colonies examined in July and August had larvae. The probable explanation of the difference lies in the fact that the larvae of *Botryllus* develop in the atrial cavity of the zooid, from which they escape to the exterior when fully developed. The larvae of *Diplosoma*, on the other hand, completing their development embedded in the basal test of the colony, certainly escape less easily, and perhaps only with the dissolution of the parent colony.

Budding

Asexual reproduction by budding may, in general, occur at any season and a high proportion of all colonies examined had buds. The process appeared to be less active, however, in August and September, both in the old generation and in the newly established colonies. In *Botryllus*, as in *Diplosoma*, exhaustion follows the intense sexual activity of the summer, and reduced asexual reproduction is here the prelude to the death of the old colony. It is surprising that budding activity was not intense in the young colonies of August and September.

SUMMARY

The populations of *Ciona intestinalis*, *Ascidiella aspersa*, *Diplosoma listerianum* and *Botryllus schlosseri* in the Old Dock of Ardrossan Harbour, Ayrshire, were studied in 1950 and 1951. Samples taken at intervals of about 4–7 weeks were used to follow growth, the progress of breeding, and the number and duration of generations.

In each species fastest growth was in May, June and early July. There was no growth in winter.

In all the species studied, except *Ciona intestinalis* for which sufficient data were not obtained, growth was several weeks later in starting in 1951 as compared with 1950.

Each species behaved as an annual, which settled as a larva, grew to sexual maturity, bred, and died, within a period of 12–18 months.

Breeding in each species was confined to a few months in the summer, and took place in the year following that in which the animals settled as larvae. *Diplosoma* may possibly also have bred at the end of its first summer.

In *Diplosoma* and *Botryllus* similar stages in the breeding cycle were several weeks later in 1951 than in 1950.

In *Ascidiella* and *Ciona* critical body sizes were found at which eggs and sperm began to fill the genital ducts. These species are protandrous; after the sperm starts to fill the sperm duct the body grows about 5 mm. before eggs pass into the oviduct.

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ON AMPULLARY TISSUE IN THE LARVA OF *POLYCLINUM AURANTIUM* MILNE EDWARDS

By D. B. Carlisle, M.A.

From the Plymouth Laboratory

(Text-fig. 1)

The larva of the compound ascidian *Polyclinum aurantium* Milne Edwards (1842) has been described by Berrill (1950), and his figures and description indicate a dissociation between the ampullae and epidermal vesicles. A re-examination of the larvae of this form shows that this is a false disjunction. This is most evident in the younger stages. Berrill describes (p. 46) a ring of eight ampullae, in the larva of *Polyclinum*, surrounding the anterior tip of the trunk, and 'numerous epidermal vesicles growing out or detached from two long strands reflected posteriorly' (p. 89).

The young larva, at the time when the yolk sac is incomplete ventrally and the atrial invaginations are just beginning (Fig. 1 *a*), already possesses fully formed sense organs and rudimentary suckers. In the epidermis there are two well-defined lateral ridges starting dorsally about one-third from the anterior end and running forward on either side of the suckers. They then run on each side of the mid-ventral line back to the base of the tail. Anteriorly these ridges swell, between and beside the suckers, to form ampullary lobe rudiments. There is no trace here of a ring of ampullae surrounding the anterior tip.

At a somewhat later stage (Fig. 1 *b*, *c*), when the larva is nearly fully formed, about four 'ampullae' have been developed on each side of the suckers by outgrowths from the ridges. Farther back the lateral ampullary ridges have budded off vesicles which remain attached to the ridges by thinner or thicker necks. In the intermediate region, dorsally and ventrally, between the ampullae and the vesicles are outgrowths which might receive either name—ampullae or vesicles. They are intermediate in position and size, and may be regarded as small ampullae or large vesicles. There is an even gradation from ampullae to vesicles. The arrangement at this stage is a pair of lateral ampullary ridges which start dorsally on either side of the branchial siphon, run forward and turn in a ventral direction beside the suckers; they then turn to run backwards on each side of the mid-ventral line and end with a dorsally reflected hook beside the base of the tail. These ridges bear outgrowths on short stalks, which, in the more posterior parts of the ridges are small and known as vesicles, but in the more anterior portions are large and known as ampullae. There is no morphological difference between them.

In the fully developed larva the lateral ampullary ridges are less obvious—perhaps more of their tissue has entered into the formation of vesicles and ampullae; some of the vesicles seem to have severed their attachment to the ridge by an attenuation of the stalk, and the difference in size between the vesicles and ampullae is more pronounced, but the gradation between them is never quite obliterated.

I wish to thank the Director of the Plymouth Laboratory for facilities for research, and Miss P. Kott for her co-operation.

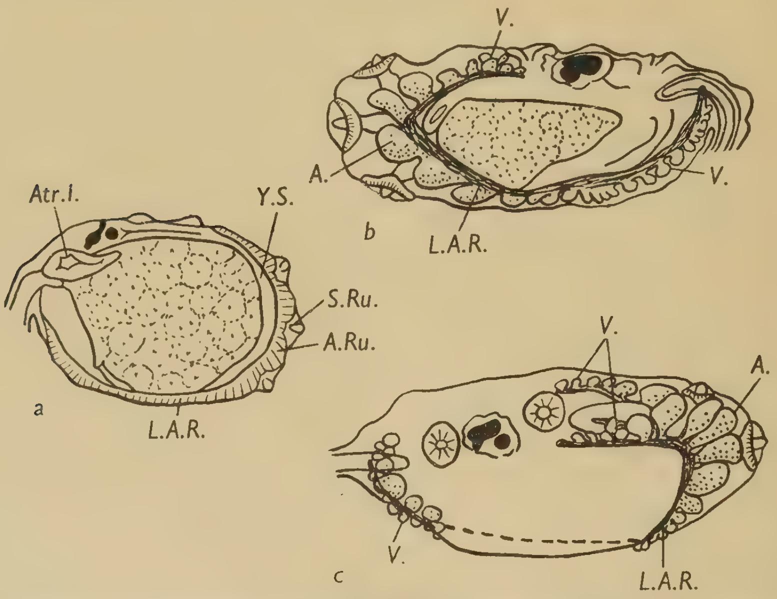

Fig. 1. Camera lucida drawings of larvae of *Polyclinum aurantium* to show the lateral ampullary ridges. *a*, a larva at the stage when the yolk sac is incomplete ventrally, from th right side; *b*, a nearly fully formed larva, from the left side; *c*, a larva at the same stage a *b*, a dorso-lateral view from the right side. *A.* ampulla; *A.Ru.* rudiment of ampulla *Atr.I.* atrial invagination; *L.A.R.* lateral ampullary ridge; *S.Ru.* rudiment of sucker *V.* vesicle; *Y.S.* yolk sac.

SUMMARY

The morphology of the ampullary lobes and epidermal vesicles of the larv of *Polyclinum aurantium* M. Ed. (Tunicata) is described anew and the tw structures are shown to be outgrowths, differing only in size and anteroposterior position, of two lateral ampullary ridges.

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OBSERVATIONS ON COMPOUND ASCIDIANS OF THE PLYMOUTH AREA, WITH DESCRIPTIONS OF TWO NEW SPECIES

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(Text-figs. 1–3)

There is still often confusion as to the identity of species of compound ascidians. This is due sometimes to a lack of published observations on the group, and sometimes to seasonal variations from the described condition. An attempt has been made to follow the growth and variation of the species in the Plymouth area throughout the year. During the course of these investigations all the species of the sub-order Aplousobranchia formerly recorded from this area were encountered, with the one exception of *Distaplia garstangi* Berrill. In addition, two new species were taken: a species of *Polycitor*, the first record of the genus in these waters, and a species of *Lissoclinum*.

L. argyllense Millar, recently described from the west coast of Scotland, was also taken from the Mewstone Ledge and from Salcombe.

No general attempt has been made to give synonymy or literature already adequately supplied by Berrill (1950) in his recent Ray Society Monograph on the Tunicata.

Conversations with Dr R. H. Millar of the Scottish Marine Biological Station at Millport, and with Mr D. B. Carlisle at Plymouth, have been most helpful and stimulating. My grateful thanks are also due to Mr F. S. Russell, Director of the Plymouth Laboratory, for his constant encouragement and advice.

Archidistoma aggregatum Garstang

Distribution. Previously taken from Salcombe and Plymouth Sound, it also occurs plentifully on the Mewstone Ledge, and so is probably present at other places around the coast.

The Colony. Berrill (1948, 1950) has described zooids 'a few millimetres long'. In June–July, when the colonies are well developed and may form carpets over the rock, the zooids extend up to 5–6 mm. in length. Clumps of two to six zooids extend from the sand-covered basal ramifying stolonial processes; the distal part of the vertical cylindrical clumps, which houses the thorax of the zooid is, however, always quite free of sand and gives the colony its typical appearance (Fig. 1 C).

Growth of the colonies takes place especially from February until maturity of the zooids in August. There is a proliferation of the posterior abdomen into the basal stolonial connexions and subsequent swelling and development of new upright clumps of zooids in the colony (Fig. 1 A, B), or addition of zooids to any already existing clump. This is similar to the asexual type of budding found in *Morchellium* and takes place not only when accompanied by a resorption of the thorax in October and November, as Berrill has suggested.

Since the development of the zooids comprising a lobe of the colony may thus occur in the basal test and the subsequent vertical system arise from this, it is unlikely that single zooids arise from the basal test and later join together (Garstang, 1891), despite the sand embedded in the test between the zooids. This sand inside the colony is very sparse and is not an unusual feature in compound ascidians. The sand-free thoracic part of the test, alone, shows any tendency to independence of the zooids. Therefore it seems possible that there is no justification for even a subgeneric group Eudistoma. The larva of a species of the genus *Eudistoma* (Berrill, 1947*b*) is identical with the larva in this species.

Polycitor searli **sp.nov.**

This species is named after Mr William Searle, who has for 56 years been fisherman and collector for the Marine Biological Association at Plymouth.

Distribution. It has been taken as yet only from Duke Rock and New Grounds in Plymouth Sound.

The Colony. The colonies are inconspicuous, of 4–6 mm. height (Fig. 1 D). They occur in rock crevasses in water of 4–5 fm. The posterior part of the colony is sand-covered. Anteriorly the test is clear and glassy and the line between the sandy and clear part of the test is very abrupt.

From 4 to 12 zooids occur in each colony and open on the surface with separate 6-lobed branchial and atrial openings. The shape of the colony varies considerably from rather narrow cylindrical specimens to a more squat circular form, and occasionally there is a constriction where the sand-covered part changes to the smooth transparent test.

The Zooids. The zooids (Fig. 1 E) vary in colour from white to red and orange, and each occupies the whole length of the colony. The atrial apertures are all directed toward the centre, and there is no common cloaca, as in a clump of *Archidistoma.* Both the atrial and branchial apertures are six-lobed. There are four rows of stigmata with six to eight stigmata in each row. The oesophagus is long and narrow, opening into an elliptical stomach (Fig. 1 F) in the posterior part of the abdomen. The stomach is smooth-walled externally but internally has four folds as in *Clavelina.* The mid-intestine after running posteriorly and dorsally opens into the enlargement of the posterior intestine. Gonads are enclosed posterior to the stomach in the alimentary loop. There are about ten pear-shaped testicular lobes, and a 3–4-celled ovary to the right of the testes. The testes do not extend posteriorly to the alimentary loop as in *P. vitreus* (Sars).

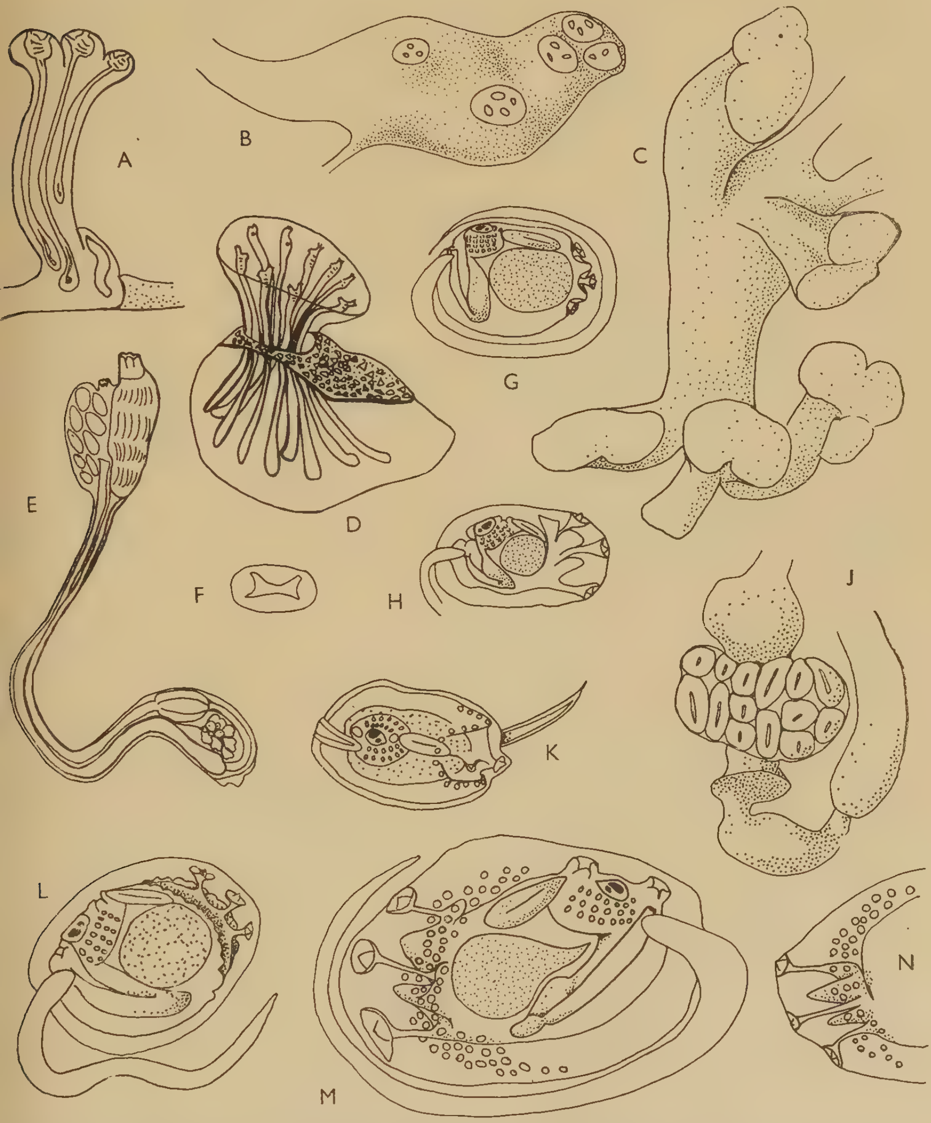

Fig. 1. A–C, *Archidistoma aggregatum*: A, section through a clump of zooids showing formation of a new zooid, ×4; B, zooids developing in a basal stolon, ×4; C, part of a mature colony, ×6. D–H, *Polycitor searli* n.sp.: D, colony, ×6; E, zooid, ×16; F, transverse section through stomach; G, young larva, ×60; H, older larva, ×60. J–L, *Sidnyum turbinatum*: J, alimentary canal, ×50; K, larva, dorsal view, ×60; L, young larva, ×60. M, N, *Sidnyum elegans*: M, larva, ×60; N, anterior portion of older larva, ×60.

The Larvae. Larvae (Fig. 1 G, H) and developing embryos appear in a brood pouch at the side of the branchial sac in June, and are present throughout July and August. The brood pouch is formed by an enlargement of the distal part of the oviduct. The larvae are small, 0·36 mm. in length without the tail. There are four rows of stigmata, a 3-celled ocellus and an otolith, three suckers and the anterior part of the larva is separated off into a horn, bearing an ampulla between each two suckers. This is similar to the condition to be described for *Aplidium proliferum* (p. 74) and *Sidnyum elegans* (p. 70). In earlier larvae the yolk material projects farther forward and the ampullae are not differentiated. The tail is twice the length of the body.

Discussion. This is the first record of a species of *Polycitor* from British waters. The lack of knowledge on its distribution is possibly, as with others, due to its small size and inconspicuous appearance. Due to the delicate and glassy nature of the test the colony is practically invisible to the naked eye, especially if the colony has been damaged and the zooids, as often happens, have been retracted into the basal part of the test. The species differs from others of the genus by the small size and delicacy of the colony and zooids. *P. vitreus* (Sars) and *P. crystallinus* Ren. are, however, the only species to have been described with any certainty. Berrill (1948) has described the larva of *P. vitreus*, but apart from the length of the tail and the number of rows of stigmata it is not similar to the larva of this present species. Nor is the brood pouch of *P. searli* constricted off in the same way as the brood pouch of *P. vitreus* (Sars).

The type specimen is in the British Museum (Natural History), South Kensington.

Distaplia rosea Della Valle

This is a common species which is extremely variable in shape and colour, ranging from pink to purple and brown. Colonies may be sessile on a spreading base or stalked. Sometimes the stalks are branched, and several stalked colonies may arise from a common base. The larvae appear in August, two to four in each brood sac. The ampullae associated with the suckers vary in each larva, and occasionally are reduced to the condition described by Berrill for *D. garstangi*. No specimens of *D. garstangi*, as characterized by a smooth stomach, were taken; but since one of Berrill's (1947*a*) distinguishing characters was the shape of the colony and all specimens of *D. rosea* are not squat as he described but often vase-shaped (cf. *D. garstangi*), it is possible that the two may be synonymous.

Sidnyum turbinatum Fleming *nec* Savigny

This name was first given to a species of compound ascidian by Savigny (1816). Savigny describes his species, however, as having six branchial lobes. Milne Edwards, in 1845, described a genus *Parascidia* with eight lobes to distinguish it from *Amaroucium*; and in 1863 Alder included, in this genus

Parascidia, the species *Sidnyum turbinatum* (Fleming, 1828; Forbes & Hanley, 1848) under the names *Parascidia flemingii* and *P. forbesii*. Thus Fleming was the first to describe a species with eight branchial lobes, and the features of the species *Sidnyum turbinatum* as it is known to-day, but the name was actually preoccupied by Savigny whose definition does not distinguish the genus from *Amaroucium*, and certainly does not include the species Fleming and Forbes described under the same name. Therefore the genus is correctly *Parascidia* Milne Edwards, and the specific name *flemingii* Alder. Since, however, the genus is known widely to-day as *Sidnyum*, that generic name is proposed as a *nomen conservandum*: *Sidnyum* Fleming. The specific name *turbinatum* applies automatically to the type species of *Sidnyum* and would therefore be retained. (For other synonymy see Alder & Hancock, 1912, pp. 19, 20, under *Parascidia forbesii* and *P. flemingii*; and Berrill, 1950.)

The Colony. The colony varies considerably in different environments. The typical condition on the shore, where it is one of the highest ascidians in the intertidal area, is that of clumps of conical colonies each containing two to four systems of about six to ten orange-coloured zooids. Colonies are joined by basal stolons and fixed in small niches in the rocks. These increase in size from February to August, when they reach a height of about 20 mm. and measure 10 to 15 mm. across the top. In August the larvae appear, developing in a brood pouch in the distal end of the oviduct, and the posterior abdomen is cut off from the thorax which is then resorbed.

Other colonies dredged from deeper waters on the Mewstone Ledge (10–15 fm.) have a more fleshy test. Zooids in these specimens vary considerably in size within a colony and vary in colour from white to orange. Large colonies also reach a maximum size (in August) of 40 mm. in height and with a corresponding increase in the number of systems, but the smaller colonies have the same dimensions as those found in the intertidal area. It may have been these larger colonies (which have the same dimensions as *S. elegans*) which led Berrill (1950) to confuse the specimens with the latter species and to describe their larvae as of *S. elegans*. The larva of *S. elegans*, as described below, is entirely different.

The Zooids. It is the zooid which, with the larvae, distinguishes the species from *S. elegans*. The branchial siphon is 8-lobed and there are occasionally four red pigment spots around the siphon, as in *Morchellium argus*. The branchial sac has from seven to nine rows of stigmata, with nine to ten stigmata in each row. The stomach has from twelve to fifteen folds, in all degrees of proliferation, but never acquiring the mulberry-like form of *Morchellium*, and occasionally showing only complete folds. The proliferations occur first on the ventral and right sides of the stomach and anteriorly. There is a prepyloric expansion of the oesophagus, a posterior stomach, and oval mid-intestine, decreasing in diameter before expansion into the posterior intestine: these features of the alimentary canal are found in all species of the genera *Aplidium*, *Morchellium* and *Sidnyum*, although they are not evident unless the gut is empty (Fig. 1J).

The Larva. The young larva has the developing ectodermal ampullae supported on two ridges anteriorly, extending ventrally to either side of the tail and continuous dorsally; the three suckers are borne on a ridge between the developing vesicles and the tail coils in a groove to the left of the suckers, between these and the epidermal vesicles on the left side (Fig. 1K, L). Later the tail is coiled around the body more loosely, the epidermal vesicles extend away from the body of the larvae, lose their connexions, and the whole larva acquires the characteristic appearance (cf. Berrill, 1950: *Sidnyum elegans* and *S. turbinatum* larvae). In the fully developed larva of 0·5–0·6 mm. there are thirteen to eighteen epidermal vesicles, and the row of vesicles from each side of the body meet dorsally. The anterior horn increases in length and in its degree of separation from the body as the larva develops. There is no specific distinction, therefore, in the two larvae described by Berrill (1950); he has described two different stages of the same species.

Sidnyum elegans (Giard)

Distribution. There are new records from Salcombe and Wembury on the shore, and Mewstone Ledge from 10 to 15 fm.

The Colony. Colonies are rounded, fleshy, from 20 to 60 mm. across, and about 20 mm. high. The larger colonies are sessile, and it is only the smaller ones which have a short, sand-covered, stalk. Berrill (1950) describes the colony as 'rose with milky patches'. The milky patches are due to the test and to the branchial and atrial siphons which are white; the rose colour is due to the branchial sac. The zooids are arranged along long common cloacal canals which, when several meet, form a common cloaca. In smaller colonies the systems are oval or elongate. The zooids are, however, closely packed in the test, and are much more dense than in *S. turbinatum*, therefore the systems are often hard to distinguish.

The Zooid. The zooid is larger than that of *S. turbinatum*. There are ten to twelve rows of stigmata with ten to fourteen stigmata in each row. The stomach is deeper, with eighteen to twenty-two folds which are only rarely broken or proliferated. The posterior abdomen varies immensely in length, as is usual with all species of this family.

The Larva. Larvae are present in the brood pouch (the distal part of the oviduct) from August to November, and therefore this is one of the longest breeding of the compound ascidians (excluding Didemnidae). The size is large (Fig. 1M, N), almost 0·8 mm. in length when fully developed. The larva is very similar to that of *Aplidium nordmanni*, but slightly smaller. There are three suckers and between them two ampullary cones, convex dorsally where they embrace the base of the suckers. Later there is an anterior horn which separates slightly at its dorsal extremity and supports the suckers and ampullary cones. The anterior horn extends varying distances dorsal to the origin of the most dorsal sucker. Sometimes there are extra ampullary cones between either the first and second or second and third cones. Plentiful epidermal vesicles which have lost their attachment with the rest of the larva are present anteriorly and arise from the ridge either side of the curled tail in the earlier embryo. These extend no farther forward than the base of the ampullary cones, unlike *A. proliferum*, where they occur around and anterior to the cones.

Morchellium argus (Milne Edwards)

The Colony. This is a common form in this area, and the appearance of the colony varies in the extreme throughout the year. Colonies described by Berrill (1950) represent only the mature forms found from the end of July to September. The thorax then resorbs and the colonies are left as flattened and rounded basal portions containing the posterior abdomina. New colonies develop in February (Fig. 2A) and consist of a central corm with a single system of about eight zooids and basal spreading stolons. The posterior abdomen strobilates into these spreading stolons which increase in size and cover a greater area; new zooids arise from the strobilated posterior abdomina and new heads appear in the spreading stolons (Fig. 2B). Strobilation of the posterior abdomen continues, and in May and June the colonies consist mainly of extremely swollen basal test (Fig. 2C) with a few rounded systems protruding. The colonies now appear as closely packed, pinkish, and very opaque, rounded masses covering large areas. In between each rounded mass, but hidden by them, there remain the connecting basal strands, themselves much swollen. Then in July the spread of the colonies stops, but they increase in height due to a growth of the zooids. There are now zooid-filled corms with sand-covered stalks which may reach a height of 150 mm. in favourable areas (e.g. Salcombe), but are usually only 2 to 3 cm. high. Larvae are then found in the brood pouches and later the thorax is resorbed and the colonies consist again of strobilated posterior abdomina. The process of asexual budding from the strobilated posterior abdomen is, therefore, in this species most exceptionally active throughout the year.

The Zooid (cf. Berrill, 1950, p. 95). The adult characters of the zooid are most consistent in this species and the stomach is quite distinctive.

The Larva. Berrill (1950) has described a larva for this species, but it is apparently undeveloped or an aberrant form, as none of the many specimens examined by me showed the same characters.

The larva (Fig. 2E) has four rows of stigmata in the branchial sac, an otolith and an ocellus, and a large yolk sac. Anteriorly, there is a horn bearing three suckers with two anterior ampullae between them (as in *Sidnyum elegans*). There are sixteen pairs of epidermal vesicles on either side of the body, arranged in a double row. In the younger larva each pair of epidermal vesicles arise from a single stalk on the anterior ridges on either side of the

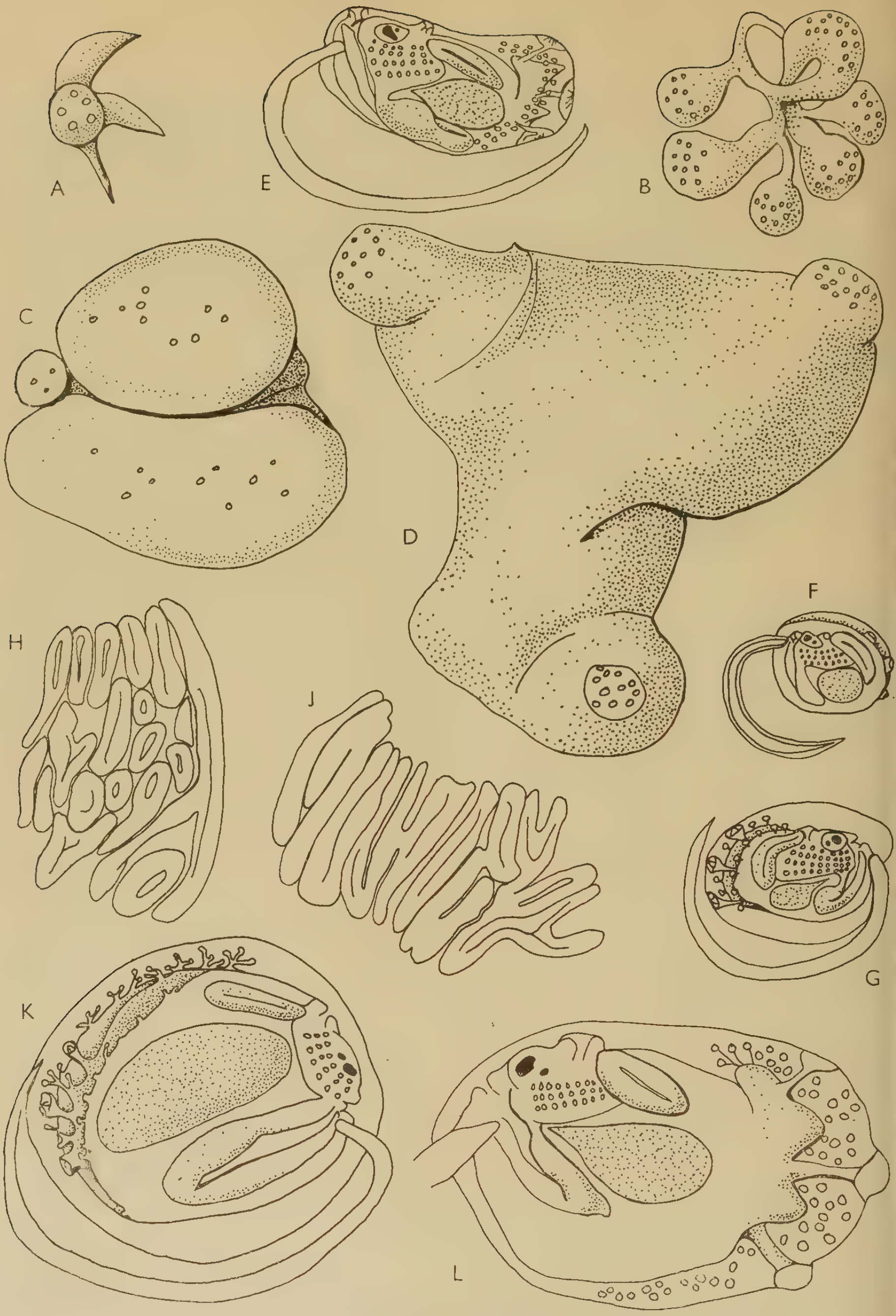

Fig. 2. A–E, *Morchellium argus*: A, young colony, February, × 1·2; B, zooids developing in spreading stolons, March and April, × 1·2; C, swollen basal stolons from which develop upright systems, May and June, × 4; D, upright systems developing, × 4; E, larva, × 60. F, G, *Aplidium pallidum*: F, young larva, × 60; G, older larva, × 60. H–L, *Aplidium proliferum*: H, '*proliferum*'-type stomach (D. B. Carlisle del.), × 100; J, '*nordmanni*'-type stomach, from the same colony as H (D. B. Carlisle del.), × 100; K, f. *nordmanni*, young larva, × 60; L, f. *nordmanni*, older larva, × 60.

coiled tail and the ridge bearing suckers and ampullae (as in *S. turbinatum*). The left and right rows of epidermal vesicles meet dorsally and lose their connexion with the body of the larva. The specimen Berrill has described is perhaps a young larva, since the suckers tend to develop more at the dorsal and ventral side of the cup which may account for the 2-lobed condition in Berrill's figure (1950, p. 96).

Aplidium pallidum (Verrill)

The Colony and the Zooid. The colony and the zooid are characteristic throughout the year.

The Larva. The larva 'is distinctive, partly because of its small size but more because of the absence of both ampullae and vesicles. Also notable is the more or less vertical position of the endostyle' (Berrill, 1950, p. 99). This is true of all the younger larvae found in the brood pouch (Fig. 2E), but in more fully developed forms (Fig. 2G), when the tail is less tightly curled around the larva, a single row of six to eight epidermal vesicles develop on each side of the body, as in *S. turbinatum* and *Morchellium argus*. These vesicles, however, unlike the larvae of *Sidnyum* and *Morchellium* maintain for longer their connexion with the rest of the larva. The larva is, as Berrill remarks, very small, which is to be expected since the zooids of this species are also small.

Aplidium proliferum (Milne Edwards)

There has been considerable confusion between this species, *Aplidium nordmanni* and *A. densum*. Thompson (1934) and Berrill (1950) have suggested synonymy between at least two of the species. The main differences for distinction exist by definition in the number of rows of stigmata in the branchial sac and the number and condition of folds in the stomach. It has been found, however, as will be shown in the discussion below, that there is no constancy, in any species, of any one of these characters; that the size of the zooids and the size and colour of the colony is the only dependable character for separation and that there are intermediate forms between these. And indeed *A. nordmanni* and *A. proliferum* were originally separated by Milne Edwards (1841) solely on the appearance of the colony. The proliferations of the stomach in the latter species, which have since been the distinguishing character, is not described (Milne Edwards, 1841, p. 287): 'l'estomac...est marqué d'une serie de plis verticaux'. It is proposed, therefore, that the species of *A. nordmanni* and *A. proliferum* Milne Edwards are forms of the same species, and that they should be known as *A. proliferum* f. *nordmanni* and *A. proliferum* f. *typicum* respectively, and that *A. densum* (Giard) is synonymous with the latter.

Aplidium proliferum (Milne Edwards) f. *nordmanni* Milne Edwards

The Colony. The colonies are thick (usually about 20 mm.) and sessile. Each colony has about three systems. The common cloaca of each system is not always central. Around the walls of the colony the test is red, but around the zooids the test is transparent and white, allowing the red of the branchial sac to show through it. The branchial and atrial siphons are white. The colony is thus in some ways similar to that of *Sidnyum elegans*, but the zooids are considerably larger and more sparsely distributed in the test.

The Zooid. Rows of branchial stigmata vary from nine to twelve with twelve to sixteen stigmata in each row. The stomach is large, varies in depth, and the number of folds (even within one colony) varies from twenty to thirty. Similarly, in their condition, the folds (again from zooids on the one colony) vary from complete unbroken through branching to completely broken up, when the stomach is given a 'mulberry' appearance (Fig. 2H, J). In a colony from Salcombe the zooids are slightly smaller, but the test is red on the outside and colourless around the systems of which there are many, and the colony is flat and investing. This, then, suggests the condition of the *typicum* form and may be considered intermediate between the two, but the numbers of folds and branchial rows conform with the *nordmanni* form.

The Larva. The fully formed larva (Fig. 2L) is the largest known and measures 1 mm. without the tail. There is an anterior horn as in *S. elegans*. It is distinguishable from the latter species only by extension farther anteriorly of the epidermal vesicles. Larvae are present in the distal part of the oviduct in August. In the younger larvae (Fig. 2K) the epidermal vesicles usually develop from the branched stalks on the anterior ridges either side of the coiled tail, as they do in all other species with epidermal vesicles. Here, as in *S. elegans*, however, there are many vesicles to each stalk, while in *Morchellium argus* there are two vesicles from the one basal stalk, and in *Aplidium pallidum* and *Sidnyum turbinatum*, a single vesicle only is borne on each stalk. In *Aplidium proliferum*, in addition to the lateral vesicles, there are similar vesicles borne on the anterior ampullae in the median line between the suckers, and these are usually very much better developed and persist longer than the lateral vesicles which sometimes do not appear to be present. The median ampullary lobes seem to be reduced in size as the larva grows older; this is also shown by Milne Edwards (1841) in his account of the development of *Amaroucium proliferum*. Therefore, it seems unlikely that the median ampullary lobes serve the same functions as the lateral paired ampullary lobes in other larvae, in consolidating the animal's hold on the substratum.

Aplidium proliferum (Milne Edwards) f. *typicum* nom.nov.

The Colony. Usually flattened ellipsoidal forms with yellowish zooids in a transparent test. The zooids are much smaller than those of *nordmanni* form, the systems are less distinct and the zooids more closely packed in the test.

The Zooid. The branchial sac has from eight to eleven rows of stigmata with ten to sixteen stigmata in each row, and from twenty to twenty-five stomach folds. These figures cover the conditions found for *A. densum* (Giard).

The Larva. The larva is similar in every respect to the larva of the *nordmanni* form.

Didemnum maculosum (Milne Edwards)

Nothing further can be said on the synonymy of this species with Michaelsen's *D. helgolandicum*. Several authors (Hartmeyer, 1923; Millar, 1949) have pointed out its similarity, but unfortunately Hartmeyer died before he had completed a comparison of the two species.

Spicules. These are from 10 to 30 μ and are as figured by Hartmeyer (1923, Taf. I, fig. 19). They are indeed similar to those found in all the didemnid and trididemnid species of this area (except *D. gelatinosum*), and are of geometrical design. They are spherical, and as the diameter of each plane through the sphere increases, so the number of points of the star increases. Therefore, looking into any aspect of the sphere, there are 1, 4, 8 (in the greatest diameter) points. In larger spicules 1, 6 and 9 points, and in smaller spicules simply 4 and 8 points (Fig. 3A).

The density of the spicules varies, but there is never at any time the same density as in *Trididemnum alleni*. They are present mainly in the surface layer of test and in the layer of test separating the thorax from the abdomen of each zooid.

The Colony. Zooids are not arranged in well defined systems, but occur sometimes in rows, or irregularly scattered in the test opening into an extensive common cloaca. The surface test is therefore joined to the basal test by the zooids and by test material around the zooids.

The Zooid. The orientation of the thorax may be in a vertical position, at right angles to the surface, or at an angle to the surface. This latter condition is associated with a greater density of spicules, and colonies of this nature are often confused with *T. alleni*. There are six branchial lobes; the ventral three lobes tend to be longer than the three dorsal lobes, especially when the thorax is inclined to the surface. The endostyle is uppermost and the ventral lobes need this extra length to turn dorsally to the surface past the thickness of the endostyle. There are four rows of branchial stigmata with six stigmata in each row. The atrial siphon is a simple opening into the common cloaca. There is a lateral spicule-forming organ either side of the thorax. The abdomen is usually smaller than the thorax. The testis has seven coils.

The Larva. The larva has four paired anterior ampullae and two suckers. In the branchial sac there are four rows of stigmata by which the larva is distinguished from that of *T. alleni*. The anterior ampullae develop from the two ridges either side of the coiled tail and show a tendency to divide so that occasionally five are found on one side of the body. In several colonies from Duke Rock the larvae have only three paired anterior ampullae. The larvae are present in the cloacal cavity and are found from March to August.

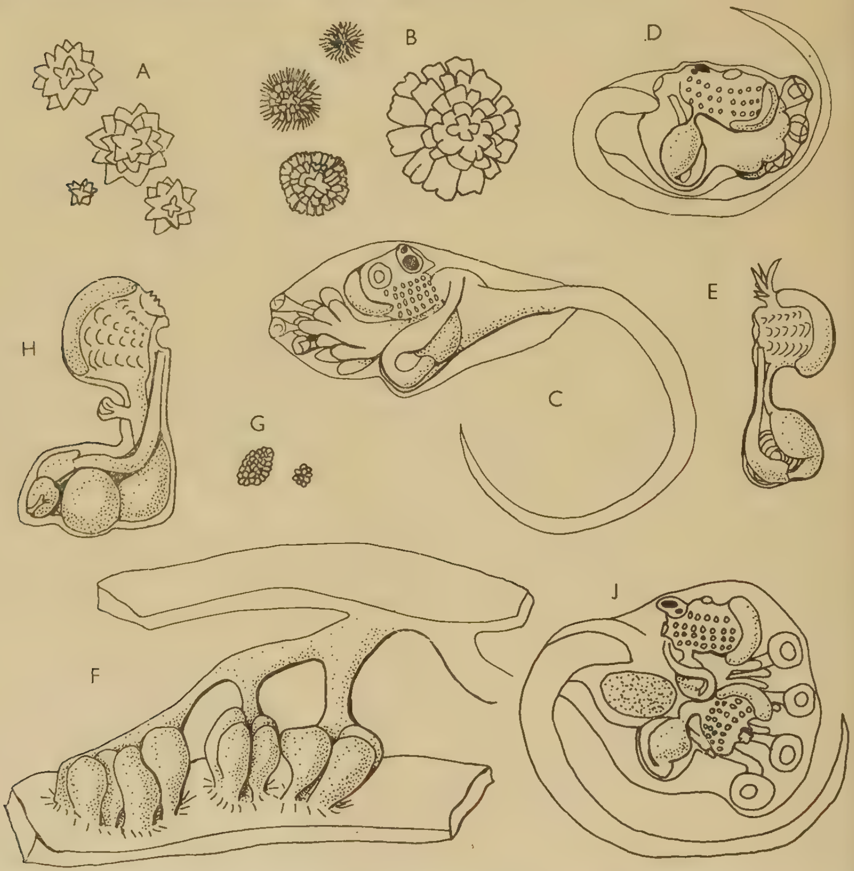

Fig. 3. A, *Didemnum maculosum*: spicules, × 400. B, C, *Didemnum gelatinosum*: B, spicules, × 400; C, larva, × 60. D, *Trididemnum tenerum*: larva, × 60. E, *Trididemnum alleni*: zooid, × 60. F–J, *Lissoclinum cupuliferum* n.sp.: F, portion of a colony showing strands of test containing zooids at the surface, × 30; G, spicules, × 400; H, zooid, × 30; J, larva, × 60.

Didemnum gelatinosum Milne Edwards

To the synonymy of this species must be added: 1912 *Leptoclinum punctatum* Alder & Hancock, Ray Society, Tunicata, VIII, Vol. 3, p. 49, and all synonyms given there, a conclusion based on the similarity of the described *L. punctatum* larva and development to those observed in the present species. The larval form of *Didemnum gelatinosum* Alder & Hancock (1912, p. 32) also conforms with the description given below.

Distribution. *D. gelatinosum* is found commonly in the intertidal zone in this area. The habitat, therefore, given by Berrill (1950) is not exclusive.

The Colony. The test is firm and gelatinous and the zooids are found in rows along an extensive common cloaca.

Spicules. These are present occasionally around the zooids, especially at the edges of the colony. The upper layer of test contains no spicules and when present they occur only in the upper part of the basal test around the base of the thorax. They are star-shaped, spherical and twice the size of the spicules of *D. maculosum* and vary from 20 to 60 μ. The rays of the larger spicules are indented, and increase in width toward the edge. The smaller ones consist of radiating spines and there are all degrees of mixing between the two kinds of ray (Fig. 3B).

The Zooid. The thorax is smaller than the abdomen and vertical to the surface of the colony. There are six branchial lobes which are small but the siphon is long. The atrial opening has no lips and the branchial sac has four rows of stigmata with six stigmata in each row. There are lateral organs opposite the second and third rows of stigmata. The abdomen is large, considerably larger than the thorax. The testis is a single lobe with the vas deferens wound eight times around it.

The Larva. These are found in the colony from March to August. They are similar to the larva of *D. maculosum*. There are, however, three suckers and usually five paired anterior ampullae, and the larvae of *D. maculosum* are smaller by about one-quarter of their length. Just as the number of anterior ampullae in *D. maculosum* is occasionally reduced to three pairs, so in *D. gelatinosum* it is rarely reduced to four pairs. Berrill (1950, p. 123) has shown a larva from a degenerating colony with four pairs of ampullae only.

Trididemnum tenerum (Verrill)

The Colony. The zooids are arranged in systems of double rows or irregular circles. The colonies are usually grey.

Spicules. The spicules are never as dense as those in *T. alleni*, or even *Didemnum maculosum*, and quite often are absent altogether. The colonies with spicules are but rarely encountered on the west coast of Scotland, and possibly the number of colonies with spicules decreases towards the north. Spicules in specimens from this area are identical with those of *Trididemnum alleni* and *Didemnum maculosum* (Fig. 3A).

The Zooid. The posteriorly directed atrial siphon is a unique and constant character. The thorax is larger than the abdomen and has eight to ten stigmata in each of the three rows, and a lateral spicule organ. There are six branchial lobes, longer than those of *D. gelatinosum*. The testis has twelve coils of the vas deferens.

The Larva. This is of the same size or larger than that of *D. gelatinosum* and has three suckers and four ampullae on each side. There are, however, several features which distinguish it from all other didemnid larvae (Fig. 3 D). The thorax is wide with three rows of stigmata and about eight stigmata in each row, and occupies the greater part of the body. The atrial siphon is well developed in the larva, and takes the adult form before the ampullae are fully differentiated from the ridges on either side of the coiled tail. The thorax occupies such a large part of the larva that the ampullae do not attain the same length as in other species. Two or three eggs develop from the ovary at the same time so that there are immense numbers of eggs accumulated in the abdomen, where they develop into larvae.

Trididemnum alleni Berrill

Distribution. This species has been taken only from the Mewstone Ledge in the Plymouth area.

The Colony. Colonies are small, brilliant white. The species is in many ways similar to *Didemnum maculosum*, the arrangement of zooids in the colony is similar and on the surface of the colony there are sometimes spicule-filled cones covering the branchial opening. These also occur in *D. maculosum*.

Spicules. These are of the same types and size as found in *D. maculosum*, but more densely packed.

The Zooid. The branchial siphon has six lobes. Berrill (1947*a*) describes this species with eight branchial lobes, but although many zooids were examined no specimens with eight lobes were seen. As found to a smaller extent in *D. maculosum*, the three most ventral siphonal lobes are larger than the dorsal lobes. The difference in size of these lobes is often so great that the inclination of the thorax to the surface of the colony does not explain the condition (Fig. 3 E). The thorax is larger than the abdomen, but altogether the zooid is the smallest didemnid zooid known.

The Larva. The larva has two suckers and four pairs of anterior ampullae. The ampullae in older larva are long and extend forwards from a much reduced base, unlike the larva of *Trididemnum tenerum* but similar to *Didemnum gelatinosum* and *D. maculosum*. The larva is about half the size of that of *Trididemnum tenerum*, and slightly smaller than that of *D. maculosum*, but the main difference here is in the number of rows of stigmata.

The whole species is most markedly similar to *D. maculosum* except in the reduced size of the zooid, which may be due to the reduction of size of colony perhaps affected by the hydroids and weed to which it is attached; and the three rows of stigmata in the branchial sac may, in turn, be a result of reduction in size of the zooid.

Lissoclinum argyllense Millar

Distribution. This species was described from the west coast of Scotland (Millar, 1950), and has since been taken in the Plymouth area at Salcombe and from the Mewstone Ledge. Thus its distribution may be expected to extend down the west coast of England, in the Irish Sea and the English Channel.

The Colony, Zooids and Spicules are as described by Millar (1950). No *larvae* were present in the colonies in March or in April.

Lissoclinum cupuliferum sp.nov.

Distribution. One large colony has been taken from the Mewstone Ledge in 15 fm. The size of the colony, the constancy of zooids and larvae found therein, and the unique character of all these, seem to justify the description of a new species although this was the only colony taken.

The Colony. The colony is greyish white, spicule-filled and where the spicules are most dense the white colour predominates. It is thick, investing. The surface and basal test, containing the greatest density of zooids, are continuous and are joined to one another by strands of test material which branch toward the surface, and enclose, at the surface, groups of four to six zooids very close together. The cavity between the basal and surface test is therefore extensive and crossed only by these strands, a typical arrangement in *Diplosoma* and *Lissoclinum*. The zooids in each clump opening at the surface of the colony are, however, held closer together in their strand of test than in *Diplosoma*, and the test is altogether tougher so that the individual zooids are not visible until, with difficulty, they are separated from the test and from one another (Fig. 3F).

Spicules. These are present in the surface and basal test and in the parts of the connective strands immediately surrounding the zooids. They are absent from the area immediately surrounding the apertures. Spicules are spherical to oval with a number of rounded protuberances so that they appear 'mulberry' like (Fig. 3G).

The Zooid. There are six small branchial lobes and four rows of stigmata in the branchial sac each with eight to ten stigmata (Fig. 3H). The abdomen is greater than the thorax. The atrial aperture is a large and simple opening into the common cloaca. The oesophagus is long with one or two buds arising from it. There is a large spherical stomach followed by posterior stomach, mid-intestine and posterior intestine as in *Diplosoma listerianum*. There is a large one-egg ovary in mature forms. So far the zooid is similar to that of *D. listerianum*, but whereas in the latter species the testis has two distinct lobes which lie against the body wall, this species has a single testis conical in cross-section, which is grooved by the vas deferens as it curves around it posteriorly and enters the testis by two branches on the left side of the larva. This grooved condition of the testis is common in *Lissocliinum*. *L. argyllense* has a vas deferens which follows the same course but the testis is not grooved. The condition here is obviously related to that in *Diplosoma listerianum*, where the lobes have become separated.

The Larva. This provides the greatest distinguishing character between this species and *D. listerianum*. The larva (Fig. 3J) is large (0·6 mm.), and exceeded in size only by the larva of *Distaplia*, *Aplidium proliferum*, *Sidnyum* *elegans* and *Polyclinum*. There are usually four suckers, but this number may be increased to five or six by the subdivision of one or more of the original four. The stalks of the suckers are bent at right angles at their distal end so that the suckers themselves face to the right. There are six anterior ampullae, three on each side. As in the larva of *Diplosoma*, however, budding is precocious, and the blastozooid is as well formed as the oozoid long before the larva is free swimming. The blastozooid forms on the right of the suckers: hence some explanation of their turning to the right. *Diplosoma* larvae are considerably smaller (0·4 mm. without the tail) than the larvae of this species.

The type specimen is in the British Museum (Natural History).

Diplosoma listerianum (Milne Edwards)

The Colony. The common test is always without spicules and is of a transparent greyish colour. The greatest variation lies in the density of zooids in the test. These are found in the branching strands of test connecting the basal to surface test, as in *Lissoclinum*, but the zooids are never arranged in such large or dense clumps. The number of zooids in a strand of test at the surface varies from one to three.

The Zooid. This is typical and invariable, being recognizable by its size and large branchial sac. The branchial siphon, however, has six lobes, not eight as in Berrill's figure (1950, p. 126, Fig. 38).

Taxonomic Characters in the Didemnid Species

In the accompanying classificatory key for the Didemnidae of the Plymouth area an attempt is made to eliminate some of the confusion existing in this family. Close resemblances may be observed between certain species of different genera, especially between the genera *Lissoclinum* and *Diplosoma*, which here are separated only by the presence or absence of spicules. This character is variable in other genera (*Trididemnum tenerum*, *Didemnum gelatinosum*) and hardly of sufficient weight to separate a genus. Other Pacific species of *Lissoclinum*, however, have more than two lobes in the testis and therefore, until relationships between these species and their east Atlantic counterparts have been compared, a union of the two genera is inadvisable. Resemblance between *Trididemnum alleni* and *Didemnum maculosum* is also very close.

Didemnidae

A. Zooids embedded in strands of test between surface and basal layers. Vas deferens not coiled.

  I. With spicules. *Lissoclinum*

    i. Testis with single lobe, ungrooved. Larva with three suckers. *L. argyllense* Millar

    ii. Testis with single lobe, grooved. Larva with four suckers and precocious buds. *L. cupuliferum* n.sp.

II. Without spicules. *Diplosoma*
   Testis two-lobed. Larva with three suckers and precocious buds.
   D. listerianum (M. Edw.)

B. Zooids embedded in the basal surface test and not clumped together by strands. Vas deferens coiled.

I. With four rows of stigmata. *Didemnum*
   i. Spicules usually absent. Larva with three suckers and five pairs of ampullae. Six stigmata in each row. Larva large. No atrial siphon.
   D. gelatinosum M. Edw.
   ii. Spicules present. Larva with two suckers and four pairs of ampullae. Larva small. Colony large. *D. maculosum* (M. Edw.)

II. With three rows of stigmata. *Trididemnum*
   i. Spicules sometimes absent. Larva with three suckers and four pairs of ampullae. Eight to ten stigmata in each row. Larva large. Atrial siphon present. *T. tenerum* (Verrill)
   ii. Spicules present. Larva with two suckers and four pairs of ampullae. Larva small. Colony small. *T. alleni* Berrill

NOTES ON THE LARVAE OF COMPOUND ASCIDIANS

It has been generally assumed that the epidermal vesicles and the ampullae of ascidian larvae are of homologous origin and function. During the present study, however, it has been observed that the anterior ampullae are of two different types—(i) the most common, with a lateral origin from two anterior ridges either side of the coiled tail in the earlier embryo, and (ii) others which arise later in the embryonic life from the median line between the suckers. The ampullae with a lateral origin persist and assist the young individual to fix itself to the substratum. The ampullae of median origin are reduced and have disappeared entirely when the larva completes its free-swimming life. The lateral ampullae occur in *Archidistoma*, *Polyclinum* and in all Didemnidae. In *Sidnyum*, *Morchellium* and *Aplidium pallidum* the lateral ridges give rise to epidermal vesicles which, to a greater or less extent, finally lose their attachment to the bulk of the larvae and undergo all degrees of multiplication from the simple row of vesicles on each side in *Aplidium pallidum* and *Sidnyum turbinatum* to a double row in *Morchellium argus* and many rows in *Sidnyum elegans*. In *Polyclinum* Mr David Carlisle, of the Plymouth Laboratory, has observed that the posterior epidermal vesicles, supposedly borne on a posteriorly directed strand (Berrill, 1950), actually arise, as do these anterior epidermal vesicles, from a continuation backwards of the anterior ridges which embrace the coiled tail (Carlisle, 1952). He has also observed that there s a continuous gradation in these larvae from the anterior ampullae which are reduced in size dorsally, and ventrally and posteriorly become vesicles attached by thin stalks to the ridges. Therefore the homology of these tructures is obvious. In *Polycitor*, *Distaplia*, *Aplidium punctum*, *A. pro-iferum* and *Sidnyum elegans* there are median ampullae which arise between the suckers and are reduced in size as the larva grows. The presence of these is not exclusive to the presence also of the lateral outgrowths which also occur in *Sidnyum elegans*, but which are transitory and less well developed in *Aplidium proliferum*. In the latter species there is an interesting condition in which epidermal vesicles also arise from the median ampullae. Assumptions on ancestry may be made from these observations: the larva of *A. proliferum* is perhaps in this respect primitive, if the whole anterior part of the ancestral larva is capable of producing vesicles or ampullae which in smaller larvae are repressed completely or limited to the lateral ridges. This argument is prejudiced by the fact that species, in other ways considered primitive, lack this median growth of vesicles, although they may (e.g. *Polycitor*) have median ampullae whose function may have been to bear the vesicles. The fact that the median ampullae are transitory lends weight to this argument. No taxonomic importance can be given to the vesicles, however, since their condition and presence is so obviously affected by the size of the larva.

SUMMARY

Seasonal variations in the form of the colony of aplousobranch ascidians in the Plymouth area are dealt with. Some of their larvae which were found to differ from existing descriptions have been re-described. Certain relationships between the larvae are discussed, especially in regard to anterior ampullae and epidermal vesicles.

A new species of *Polycitor*—*P. searli*—and a new species of *Lissoclinum*—*L. cupuliferum*—together with their larval forms, are described.

The species of the family Didemnidae and their relationships are discussed.

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INNERVATION OF THE HEART OF *LIGIA OCEANICA*

By J. S. Alexandrowicz

From the Plymouth Laboratory

(Plate I and Text-figs. 1–3)

During my stay at the Plymouth Laboratory in 1931 I had an opportunity of investigating the innervation of the heart of *Ligia oceanica*, and of making some experiments on the function of the ganglionic trunk which constitutes the main element of its innervation. In a short note describing these experiments (Alexandrowicz, 1931) it was stated that a description of the arrangement of the nerve elements would appear in a short time. However, on later examination of the preparations it seemed to be desirable to verify some of the findings, but the same animal was no longer available. Another isopod, *Mesidotea entomon*, with which I tried to continue the work during my visit to the Marine Biological Laboratory in Hel, proved to be much less suitable for this kind of investigation despite its larger size. Later events, unfavourable for scientific work, caused the loss of all my preparations and a further delay. It is only now, thanks to the chance of working again at the Plymouth Laboratory, that I have been able to resume my investigations.

I wish to record my gratitude to Mr F. S. Russell, F.R.S., for his kind help in preparing the manuscript.

METHOD

The observations recorded below were made on preparations stained with methylene blue, either by injection or by immersion of the tissues, or by a combination of the two methods (for details see Alexandrowicz, 1932, 1951). None of the methods or the various modifications, such as changing the concentration of the solutions, the temperature, or the pH have given fully satisfactory results since certain nerve elements, e.g. the ganglion cells in the main trunk, stain but rarely, while others, such as the nerves running alongside the aorta, stain fairly well in the part of their course near the heart but not at all at their origin from the ganglia in the anterior region of the animal. The latter phenomenon may be partly due to the effect of the secretion of the digestive glands which hinders the staining. It is therefore advisable when dissecting the animals to remove these glands as quickly as possible and to wash the tissues thoroughly with sea water. It is, however, difficult to prevent the organs in the anterior region of the animal from being contaminated by the secretion of the glands.

The heart can be exposed either from the ventral or from the dorsal side.

The first way is much the easier. It is sufficient to cut out the dorsal body wall and fix it to a paraffin plate with the ventral side turned upwards: the heart then appears in view partly covered by gonads which should be removed. In order to expose the nerve elements situated on the inner surface of the heart tube the ventral wall of the latter must be cut through along its median line. This should be done under a dissecting microscope with fine scissors, and it is not as difficult as it might appear owing to the fact that the walls of the heart are attached to the surrounding organs in such a way that they do not collapse. It is also very convenient both for staining and for observation that the ventral wall of the heart remains wide open after it is split, leaving the inside of the heart clearly visible. In *Mesidotea entomon* the heart tube is flaccid and it is much more difficult to split it and observe the inside.

A serious inconvenience to observation of the heart nerves in *Ligia* is due to chromatophores containing a pigment which does not dissolve in alcohol, so that the pigmented terga must be removed. This is the most difficult manipulation in the whole later procedure. It can be done best after the fixation when the preparations are being washed in water. The pigmented cells may also be present in the pericardial diaphragm and it is not possible to remove them at this spot. It is therefore preferable to choose animals which are less pigmented.

Access to the heart from the dorsal side is much more difficult, since it is necessary to cut out the terga one after another and to remove the dorsal muscles covering the heart. This is a tiresome operation, but this method exposes the dorsal surface of the heart and gives a view of its nerves from an additional aspect.

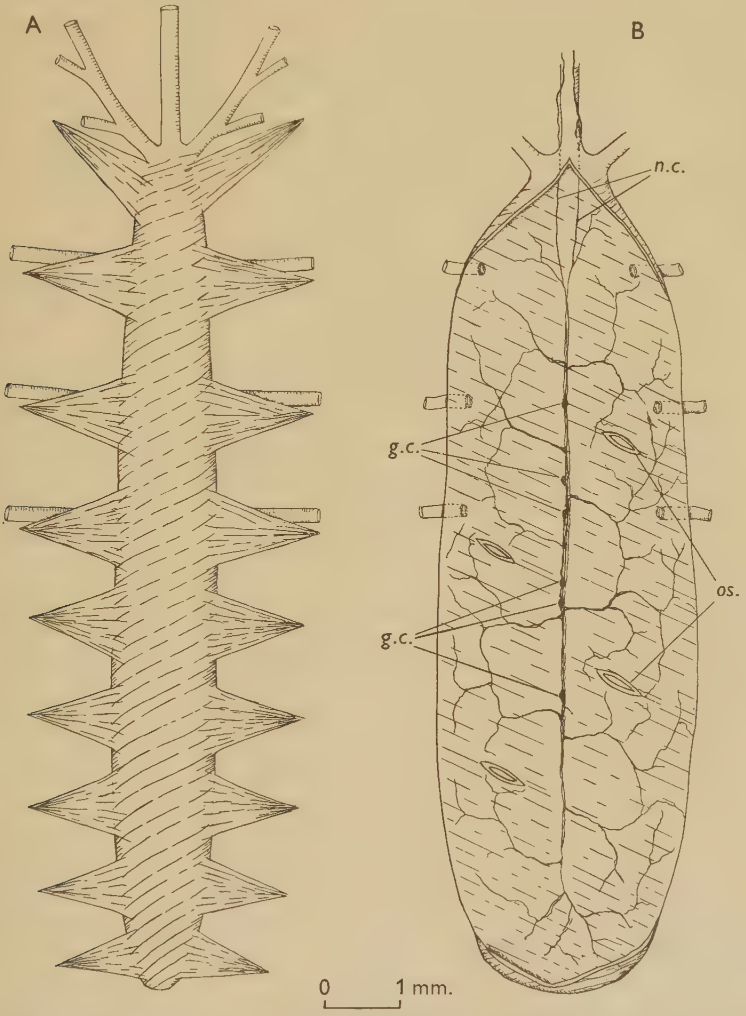
OBSERVATIONS

On the Anatomy of the Heart of Ligia oceanica

The heart of *L. oceanica* is shaped like a tube which in the largest specimens is about 15 mm. long. It is composed of a single layer of muscle fibres turning in right-handed spirals and of a thin coat of connective tissue (Text-fig. 1A; Pl. I, figs. 1, 3). On the inner side of the muscle layer there is a plexus of strands of connective tissue elements composed mostly of cells with highly refringent inclusions, presumably of fatty material (Pl. I, fig. 2).

The heart wall is pierced by four ostia situated alternately on either side as shown in Text-fig. 1B. Delage (1881) and Gordon Hewitt (1907) state that there are two only, but I have always found four of them, and they appear so distinctly in preparations in which the heart wall has been stretched (Pl. I, fig. 3) that there can be no doubt on this point. It may be mentioned that on the muscle fibres bordering the ostial orifices some cells can be observed staining deeply with methylene blue. These are mesenchymal elements differing in appearance from those arranged in the plexiform strands on the inside of the heart.

Nine arteries arise from the heart. One called the aorta runs forward in the mid-line, the others are four paired arteries situated in the anterior half of the heart as shown in Text-fig. 1 A.



Text-fig. 1. *Ligia oceanica*. A, view of the heart from the ventral side with its arteries and alary muscles; B, view of the heart cut in the mid-line of the ventral wall and stretched with the inner surface turned upwards. *g.c.*, ganglion cells in the main trunk (ganglionic trunk); *n.c.*, nerves connecting the local system with the central nervous system; *os.*, ostia.

The ventral wall of the heart is connected with the pericardial diaphragm, horizontal septum reinforced by muscles of triangular shape which may be alled 'alary muscles' as in insects. Connective tissue fibres attached to the orsal and lateral walls suspend the heart tube in the pericardial sinus, thus acting as the 'ligaments of the heart', a name given to similar elements in decapods.

As far as could be ascertained the presence of the alary muscles has not been mentioned in works on the anatomy of Isopoda, but on whole mounted preparations they can be clearly seen (Pl. 1, fig. 1). There are nine pairs of these muscles situated as shown in Text-fig. 1 A, the first four a little more widely spaced from each other than the remainder. At the apex of each triangle the converging muscle fibres pass into a slender tendon attached to chitinous ridges of the anterior borders of the terga. Towards the basis of the triangle the muscle fibres pass into the connective tissue of the pericardial diaphragm.

Nerves of the Heart

As in other groups of crustaceans in which the innervation of the heart has been investigated (Alexandrowicz, 1932, 1934) there are in the Isopoda three systems of nerve elements connected with the heart, viz. (i) a local nervous system, (ii) nerves connecting the local system with the central nervous system, and (iii) nerves of the arterial valves (Text-fig. 1 B). There are, moreover, nerve elements which, though not connected directly with the heart itself, have certain relations with its function, viz. the nerves of the alary muscles and the nerves of the pericardium and the heart ligaments.

Local Nervous System

The local nervous system consists of neurons, the cell bodies of which lie in a nervous trunk situated on the inner surface of the dorsal wall and which may therefore be called the ganglionic trunk of the heart. It runs in the midline for a great part of the length of the heart tube, being thickest along the middle region and tapering towards both ends. The whole of it is shown in Text-fig. 1 B, and parts of it in Pl. I, figs. 1–4.

Ganglion Cells

I have already given brief mention of the presence of ganglion cells in the heart of *L. oceanica* in my paper of 1931. In 1934, S. Suzuki stated that in the heart of *L. exotica* six nerve cells are present, but as they were found on sections stained with non-specific methods this writer could not observe much of the innervation of the heart in this species as well as in two other isopods (*Tylos granulatus* and *Porcellio scaber*) investigated later (Suzuki, 1935; Tanita, 1939).

In methylene-blue preparations the nerve cells rarely stain and it is surprising to see no trace of them even in many preparations in which the nerve trunk and its branches are very well stained, although this whole system apart from a few elements of different origin is derived from the processes of these cells. They are six in all, situated at various distances from one another as shown in Text-fig. 1 B. In methylene-blue preparations they appear in varying numbers and only exceptionally are all of them seen together; therefore, in order to be certain that this number is correct I verified it on serial sections tained with ordinary methods.

Very few details of the cells can be observed, not only because they are visible merely after prolonged staining when the nerve elements begin to deteriorate, but also because of their situation in the trunk among the nerve ibres as seen in Pl. I, fig. 2. Such a picture as in Pl. I, fig. 4, showing a cell not covered by the fibres and not obscured by surrounding connective tissue, s very seldom met with. When a cell is only faintly stained a largish nucleus may be seen and fine dark blue granules in the cytoplasm. Sometimes the presence of a cell can only be recognized by these characteristic granulations. In a few cases fine nerve fibres have been observed in the vicinity of a cell, suggesting the presence of a basketwork around it, but this is much less distinct than in the ganglion cells found in other groups of crustaceans.

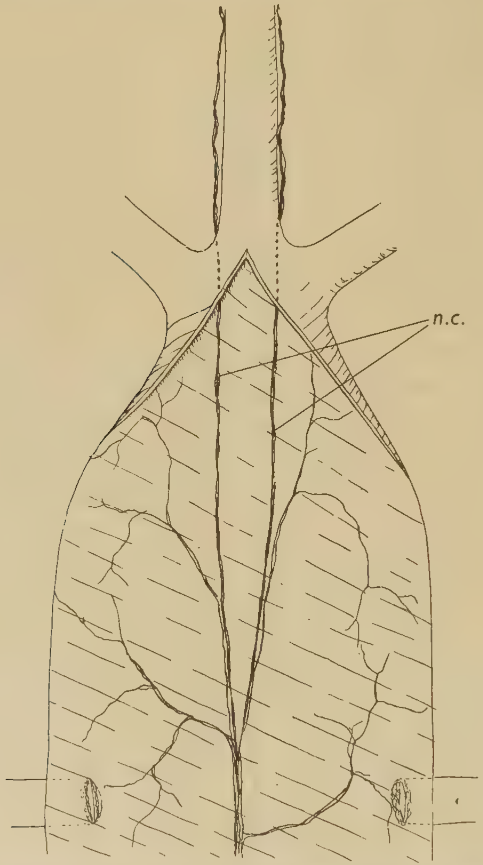
The processes of the cells stain badly, not only the shorter ones but also the axons at their origins. This feature, not uncommon in other nerve elements in arthropods, impedes the direct observation of the course taken by the axons belonging to each neuron of the ganglionic trunk; some evidence, however, may be gained from the composition of this trunk. It contains several fibres of stout calibre and a few thinner fibres, and it can be stated that in the middle portion of the trunk the stouter are six in number. This points to the conclusion that the axons of all the nerve cells are present here and it can also be assumed that the axons of the anterior cells are directed backwards and that those of the posterior cells run in the opposite direction.

The ganglionic trunk gives off branches which pass on to the walls of the heart. Two of the branches originating in the bifurcation of the anterior end of the trunk take a symmetrical course, diverging obliquely from one another. These are the branches which are joined by the nerves from the central nervous system. The branches arising from the trunk distribute their fibres over the whole heart as shown in Text-fig. 1 B and Pl. I, fig. 1, and are interconnected by anastomoses. No special endings on the muscles can be seen.

It can be noticed that the branches springing from the main trunk are composed of more than one fibre each (Pl. I, fig. 4) and that these fibres originate from different elements of the trunk. It has not been possible to trace them further, but it may be inferred that in the same portion of the heart the muscles are innervated by more than one neuron of the local system and the presence of the anastomoses between the branches affords supporting evidence for this assumption.

Nerves Connecting the Local System with the Central Nervous System

The connexion of the local system with the central nervous system is established by a pair of nerves running alongside the aorta. They associate with the fibres innervating the valves of the aorta and this makes it difficult to trace them, but on approaching the heart they separate from other fibres and, piercing the dorsal wall of the heart near its anterior end, pass on to its inner surface (Text-fig. 2). Meeting here the two anterior branches of the ganglionic trunk they run along with them into the trunk itself. These nerves appear to be composed of more than one fibre each and at some places three of them could be seen, but owing to the size of the nerve elements in *Ligia* more exact observations are difficult. The further course of these fibres in the ganglionic trunk and their connexions could not be discerned. It may be inferred only that the finer fibres seen at some places in the trunk belong to these nerves. I was also unsuccessful in my endeavours to establish the origin of these elements. As the nerves accompanying the aorta can be followed up into the first thoracic segment and even beyond its anterior border it seems probable that they arise from the suboesophageal ganglion



Text-fig. 2. Anterior part of the heart of *Ligia oceanica* from a preparation stretched as in Text-fig. 1B. *n.c.*, nerves connecting the local system with the central nervous system.

but, as I have said before, the staining of the nerves in that region has always been unsatisfactory. It is also not improbable that some fibres join these nerves during their course along the aorta, but no precise evidence could be established supporting this contention.

Suzuki believed that two pairs of nerves pass on to the dorsal wall of the heart from the abdominal ganglia and, moreover, reported the presence of small nerve cells on these fibres. I could not find such nerves in methylene-blue preparations, unless they are those which terminate on the pericardium and on the ligaments of the heart. As for the cells observed by Suzuki they were probably nerve cells of the muscle receptors, since these organs are also present in *Ligia* and are situated, as in decapods and stomatopods, on the dorsal muscles of the thoracic and abdominal segments at the place corresponding to that in which lie the cells seen by Suzuki.

Nerves of the Arterial Valves

All the arterial vessels arising from the heart are provided with valves the muscles of which receive their innervation from a special system of nerves.

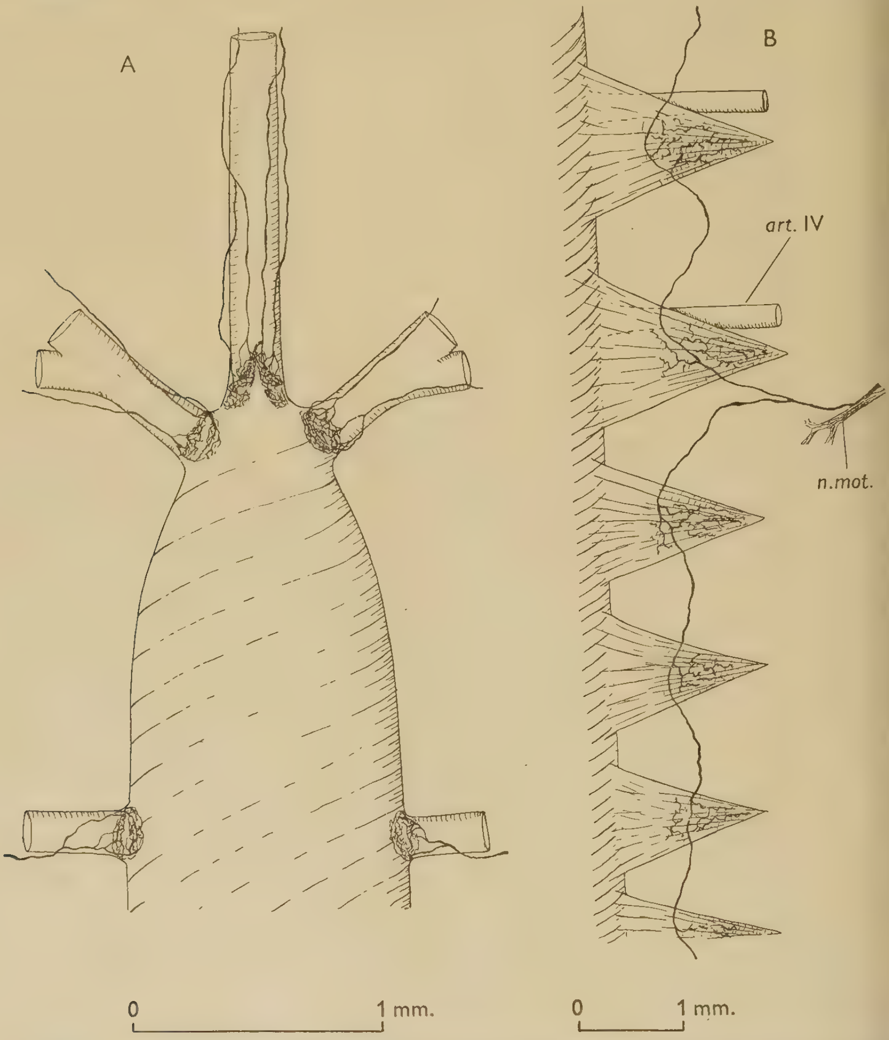
The nerves for the valves of the aorta run down this vessel and originate somewhere in the most anterior ganglia of the central nervous system. Approaching the heart they divide into several branches, giving off numerous ramifications expanding on the valves which are apparently complicated in structure, having two main and two additional flaps.

The valves of the first pair of lateral arteries receive their innervation from two fibres. One of them runs along the anterior wall of this vessel having an antero-lateral course, the other having a more transverse direction and, near the valve, lying close to the posterior wall of the artery (Text-fig. 3 A). Each of them associates more proximally with branches of motor nerves. The nerves for the valves of the following three pairs of arteries run from the ventral ganglionic cord with the motor fibres supplying the dorsal muscles of their respective segments. Owing to the different appearance of the nerves of the valves it may be assumed that they are of some special kind and that, apart from taking the same route as the motor fibres, they have nothing in common with the latter.

The distribution of the nerve fibres in the valves gives a characteristic picture of very abundant, densely arranged, tortuous ramifications. They are confined to the valves and when even nothing but the nerves are stained the outlines of the valves are well defined by their endings.

Nerve of the Alary Muscles

The nerve supplying the alary muscles runs from the central nervous system with the motor nerve of the dorsal muscles and, although its proximal course and its origin could not be traced, it may be assumed as for the nerves of the valves that it does not belong to the same category as the motor nerves of the ordinary muscles. It comes near the heart behind the fourth (last) pair of arteries and bifurcates here into branches running in opposite directions (Text-fig. 3B). They have a characteristic sinuous course passing across the dorsal sides of the alary muscles and sending to them short fibres ramifying in a pattern differing from that seen in ordinary muscles. The posterior branch is easily followed in its course to all posterior alary muscles. As to the anterior



Text-fig. 3. A, anterior part of the heart of *Ligia oceanica*, showing the nerves of the valves of the aorta and of the two first pairs of arteries; B, innervation of the alary muscles. *n.mot.*, motor nerve from which the nerve of the alary muscles is separating; *art.* IV, artery of the fourth pair.

branch I am not quite certain whether it supplies all the four anterior muscles. This uncertainty is to a great extent due to the presence in this region of the nerves of the arterial valves which complicate the picture. I tried to find out f the innervation of the valves is not in some way linked with that of the alary muscles, but no convincing evidence of such a connexion could be obtained.

Comparison of the Innervation of the Heart in Isopoda with that in other Crustacea

On comparison of the innervation of the heart of isopods with that of the two groups of crustaceans in which it is better known, i.e. in decapods and stomatopods, it becomes obvious that in all these animals it is built up on the same lines: there is everywhere a local nervous system consisting of neurons the cell bodies of which are situated in the heart itself and the processes of which spread over all its muscle fibres. The number of these neurons (six) in *Ligia oceanica* is smaller than in other species investigated (sixteen in *Astacus fluviatilis*, nine in other Macrura, in Anomura and Brachyura, sixteen in Stomatopoda). The nerves connecting the local neurons with the central nervous system show fewer details in *Ligia* than in the other Crustacea. In the latter these nerves, called ‘dorsal heart nerves’, have been found to contain two kinds of fibres one of which is represented by three thicker elements. As mentioned before, it was neither possible to state whether in *Ligia* they are of two sorts, nor what might be their number.

The innervation of the arterial valves has much in common in all these crustaceans, being in all of them independent of the local system, and in all animals the valves of the paired arteries have a segmental innervation, whereas the valves of the anterior aorta receive their supply from a nerve or nerves running along this vessel.

This nerve running down the aorta was the first to be discovered to have a relation with the heart in crustaceans. As it had been known for many years by the name of *nervus cardiacus*, I left this term when describing the innervation of the heart in decapods, adding only the word ‘anterior’ although it proved to end in the valve and not to pass farther into the heart. It may now seem inconsistent not to use the same term for the homologous elements in the Isopoda. The reason is that in the latter there are other nerves following the same route and which deserve even more the name anterior nerves’ of the heart since they pass into its ganglionic trunk. They are most probably homologous with those nerves which in decapods and stomatopods were described as *nervi cardiaci dorsales*, but again, because of their topography, the latter erm does not fit them. As for the term ‘regulator nerves’, also suggested by me previously, it may be objected that it is not advisable to derive a name of an organ from a function which is only probable. There is an obvious need of a unified nomenclature and for the adoption of terms which could be applied generally, taking nto account the fact that homologous elements may have different topographical rrangements in different animals.

In both the Isopoda and the Stomatopoda the nerves of the arteries in any one segment are independent of those of the neighbouring segments. In *Ligia* even the valves of the same pair of arteries have completely separate nerves, whilst in *Squilla* they are connected by anastomoses.

The presence of the alary muscles both in isopods and stomatopods and their obviously similar function furthering the circulation in the big blood sinuses would suggest that there should be common features in their nerve supply. As to stomatopods, it has been stated that their alary muscles are innervated by branches belonging to the system of median connectives generally called 'unpaired nerve' (Alexandrowicz, 1952). Such median connectives are present in Isopoda too, but as yet I have been unable to find out their peripheral expansion and to state whether the origin of the nerves of the alary muscles can be traced to them. This problem, as well as that of the innervation of the pericardium and the heart ligaments, might perhaps be solved by further investigations.

As regards the function of the nerve elements in the heart of *Ligia* it could be shown in the experiments previously recorded that the action of the heart is ruled by the local nervous system, since by severing this trunk different rhythms of pulsations were produced in different parts of the heart. As to the nerves connecting the heart with the central nervous system, it may be supposed that they have a regulating function carrying accelerating and inhibiting impulses.

SUMMARY

In the heart of *Ligia oceanica* (Crustacea, Isopoda) three systems of nerve elements have been distinguished: (i) a local system, (ii) nerves connecting the local system with the central nervous system, and (iii) nerves of the arterial valves.

The local nervous system of the heart is made up of six neurons which form a ganglionic trunk situated on the inner surface of the dorsal heart wall. The branches arising from this trunk expand over all the muscle fibres of the heart.

The nerves connecting the heart with the central nervous system run down the aorta and, piercing the heart wall, join the ganglionic trunk. The same route along the aorta is taken by the nerves of its valves. The valves of the four paired arteries are supplied by segmental nerves. The nerves of arterial valves have no connexion with the local nervous system of the heart.

It has been found that the pericardial diaphragm is strengthened by nine pairs of triangular 'alary' muscles. They have a particular innervation of their own, belonging neither to any of the systems of heart nerves nor to the category of motor nerves of the ordinary muscles.

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EXPLANATION OF PLATE I

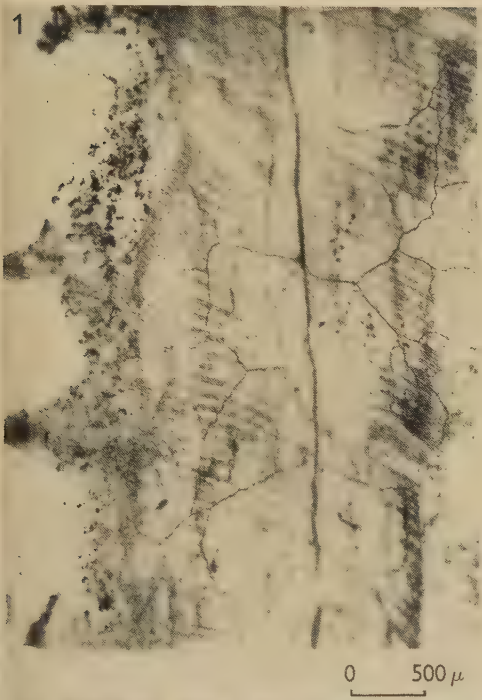
All photomicrographs have been made from preparations of the heart of *Ligia oceanic* stained with methylene blue, fixed in ammonium molybdate and mounted in xylol dammar The heart wall is shown from its inner surface.

Fig. 1. Part of the heart with the ganglionic trunk and its branches. Two alary muscles ar seen on the left side. The dark spots on the left side of the figure are the pigmented cells

Fig. 2. The ganglionic trunk and strands of connective tissue elements. *g.c.*, ganglion cell.

Fig. 3. The ganglionic trunk and the muscle layer of the heart with an ostium. The fibre crossing the ostial orifice belong to one of the ligaments of the heart and are situate on the exterior side of the heart wall.

Fig. 4. Nerve cell of the ganglionic trunk. The nerve branching from the trunk has two root proving its composition of two fibres.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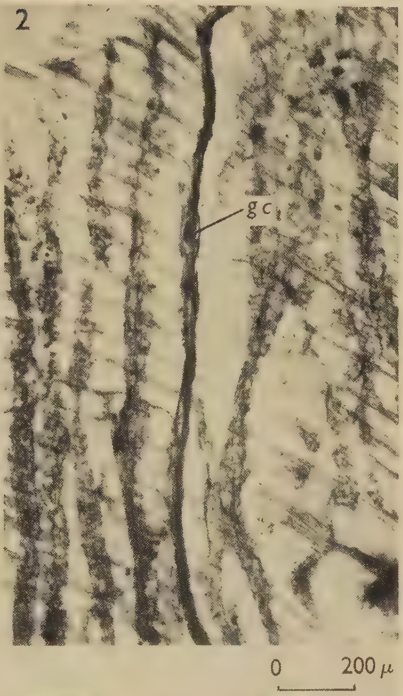
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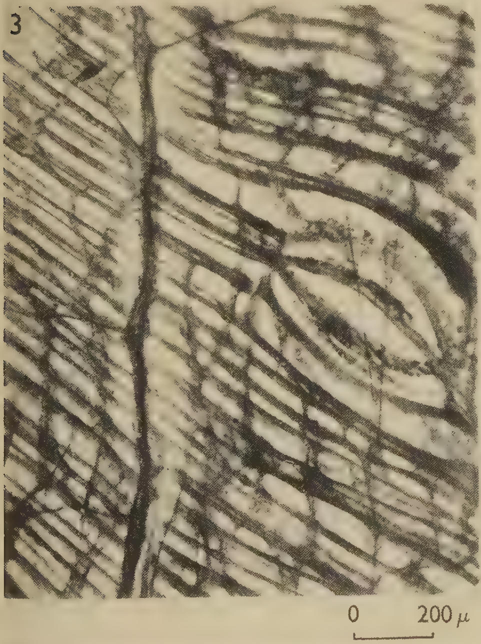
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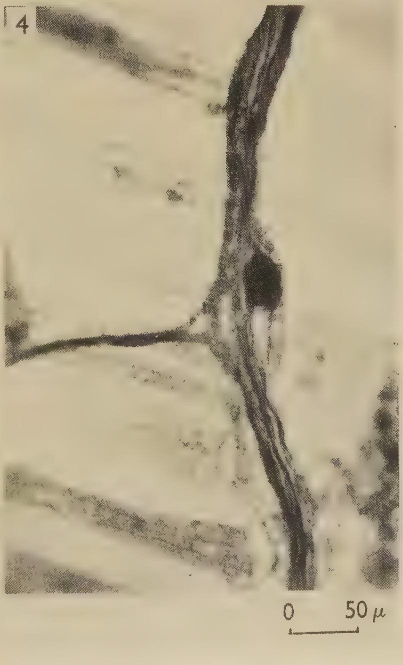
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N THE USE OF ANTIBIOTICS FOR ISOLATING BACTERIA-FREE CULTURES OF MARINE PHYTOPLANKTON ORGANISMS

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(Text-fig. 1)

n the course of some studies on the kinetics of growth and the biochemical ctivities of a marine diatom it became desirable to obtain bacteria-free ultures. The classical method of obtaining such pure algal cultures involves ither repeatedly washing single cells in sterile medium or obtaining discrete acteria-free algal colonies by growth on a solid medium. Both these methods ave been widely applied to fresh-water species by Pringsheim (1946) and thers, whilst Chu (1946) has used both methods with the marine diatom *itzschia closterium* (Ehrenberg) Wm. Smith forma *minutissima*.

The bacteria associated with algae (especially diatoms) are normally very enaciously attached, and some (e.g. the blue-green algae) may penetrate the elatinous sheaths. This characteristic of the bacteria may make a washing rocedure impractical. Chu found it necessary to make a minimum of six nitial subcultures in sterile medium under conditions that favoured algal ather than bacterial growth (low organic content of medium, high light ntensity and low temperature) before selecting single cells for washing in terile medium. It seems probable, therefore, that the washing technique will emove free-living but not attached bacteria (cf. the results of Zobell & Allen 1935), who showed that more than half the bacteria in the sea will attach hemselves to solid surfaces so tenaciously as to resist being washed off with unning water). The plating technique does not seem to be widely applicable o marine species. Some thirty species have been tried during the present tudies including representatives of the groups Cryptophyceae, Chrysohyceae, Chlorophyceae, Dinophyceae and Bacillariophyceae. Only two of he Chlorophyceae (*Chlorella* sp. and *Chlamydomonas* sp.) and one Bacillariohyceae (*Nitzschia closterium* f. *minutissima*) grew on Erdschreiber medium olidified with 1·5 % agar. Numerous workers have investigated chemical and hysical treatments in the hope of finding one that is bactericidal and which s tolerated by algae. Zobell has attempted to use heat, ultraviolet irradiation nd various chemical bactericidal compounds (Zobell & Long, 1938). Of the atter acriflavine proved to be bacteriostatic in concentrations that did not greatly interfere with the growth of diatoms. Similarly, Gerloff, Fitzgerald & Skoog (1950) have used ultraviolet irradiation to obtain bacteria-free cultures of the blue-green algae. Both these methods, however, depend upon a subsequent extinction dilution procedure.

The use of antibiotics seemed to offer a possible method. Fish (1950) employing exposure to massive doses of penicillin made some preliminary experiments with a green flagellate and an *Enteromorpha* culture. He was able to show that pre-treatment of the algae in 5000 units/ml. penicillin followed by subculture into a peptone broth (at a resultant concentration of 500 units of penicillin per ml.) prevented the growth of the bacterial flora for periods of up to 7 days and that the motility of the flagellate in Erdschreiber medium was apparently not affected by the presence of 1000 units/ml. of penicillin. In addition, by holding zoospores of *Enteromorpha* in a similar massive concentration of penicillin he was able to obtain a culture with no free-living bacteria in the medium. The flagellate was not subcultured in the absence of penicillin and subsequently tested for sterility nor was the algal material of the *Enteromorpha* submitted to sterility tests. In principle, however, the method seemed to offer a possible technique and these notes report some further work which has been directed towards extending the previous studies.

This work has been done in the laboratories of the Marine Biologica Association at Plymouth. I wish to record my thanks to Mr F. S. Russell F.R.S., for making freely available all the facilities of the laboratory, to Dr H. W. Harvey, F.R.S. and Dr Mary Parke for their continued interes and encouragement and for many helpful discussions and to the latter for supplying the cultures of phytoplankton organisms.

EXPERIMENTAL

Materials

Penicillin was obtained in a sterile condition as the crystalline sodium sal of 90% purity. Powdered sterile streptomycin (calcium chloride) was used Solutions of required strength were made up in sterile water with asepti precautions immediately before use.

Bacteriological Methods

Several media have been used for sterility testing. Their composition i detailed below.

Peptone sea water: 0·5% bacto-peptone, 0·01% ferric phosphate dissolve in 75% sea water.

Peptone sea water agar: as for peptone sea water plus 1·5% powdered agar

Casein sea water agar: 0·05% bacto-peptone, 0·05% soluble casein, 0·05% soluble starch, 0·1% (v/v) glycerol, 0·02% dipotassium hydrogen phosphate 1·5% agar dissolved in 75% sea water.

The media were made up in aged filtered sea water diluted to 75% with istilled water and were sterilized by autoclaving for 30 min. at 5 lb. pressure.

Plate cultures were inoculated by pouring, care being taken to cool the agar 42° C. before addition. All cultures were incubated at 22° C.

hytoplankton Cultures

Throughout these studies, normal bacteriological techniques were used to revent contamination. Organisms were cultured in either plugged 100 ml. onical Pyrex flasks or plugged 25 × 150 mm. rimless Pyrex tubes.

The *Nitzschia closterium* forma *minutissima* used in these studies was a sub- ulture from Allen's original strain which had been maintained in Miquel edium. The other organisms were obtained by subculture from stocks aintained in normal Erdschreiber medium.

During these experiments the *Nitzschia* was maintained in sterile enriched a water. This was prepared by diluting aged filtered sea water to 75% with istilled water and enriching with 30 mg. nitrogen (as sodium nitrate) and 0 μg. manganese (as manganese sulphate) per litre. This was sterilized by toclaving for 30 min. at 5 lb. pressure. After cooling it was further enriched ith 3 mg. phosphorus (as disodium hydrogen phosphate), 8 mg. silica (as dium silicate), and 100 μg. iron (as ferric citrate: Rhoda, 1948) per litre. he latter additions were made aseptically from sterile solutions of the quired strength.

Other organisms were cultured in sterile Erdschreiber medium prepared y enriching 75% sea water with 0·03% sodium nitrate and, after sterilizing above, enriching aseptically with 0·003% disodium hydrogen phosphate d 5% (v/v) soil extract. The latter was prepared by extracting soil with an qual weight of water by heating in the autoclave for 30 min. at 10 lb. ressure. After clarifying the extract by centrifuging it was finally sterilized y autoclaving for 15 min. at 30 lb.

he Observations

At low concentrations penicillin is essentially bacteriostatic towards those cteria which depend, for growth, upon the assimilation of glutamic acid ther than upon its synthesis. Such organisms tend to be Gram positive hereas the majority of marine bacteria are Gram negative (Zobell, 1946). The chnique of exposure for short periods to massive doses of antibiotic seemed presuppose that under these conditions it may exert some bactericidal ect. There is some evidence that penicillin exerts a killing effect and may sorganize the ribonucleic acid metabolism of the cell (for references on ode of action of penicillin see review by Peck & Lyons, 1951), but un- rtunately the sterility tests used by Fish were conducted in the presence of concentration of antibiotic that might, in any case, be expected to be cteriostatic. Information was therefore required on the persistence of the antibiotic effect of penicillin (or its breakdown products) in sea water. Varying amounts of penicillin were added to sterile peptone sea water and stored in the light at room temperature. Inoculation of these with 1 ml. of a crude diatom culture at intervals showed that after 16 days' storage the inhibitory effect on bacterial growth of the original additions of 100 units/ml. was still very considerable.

Bearing these results in mind the effect of various concentrations of penicillin on the growth in peptone sea water of the bacterial flora associated with a crude diatom culture was investigated. The results shown in Table I are typical of those obtained.

TABLE I. EFFECT OF PENICILLIN ON THE GROWTH IN PEPTONE SEA WATER OF THE BACTERIAL FLORA ASSOCIATED WITH A DIATOM CULTURE

Concentration of penicillin (units/ml.)	Days incubation before growth first visible (turbidity)
Nil	2
5	3
10	4
50	8
100	>21
500	>21
1000	>21

To tubes of peptone sea water were added various concentrations of penicillin. Tubes inoculated with 1 ml. of a crude culture of *Nitzschia closterium*. Observed daily and day when growth first seen in each tube (turbidity) noted.

Concentrations of 100 units/ml. and over inhibited bacterial growth in this medium for over 3 weeks. The correspondence between these results and those obtained by Fish on a sample of sea water suggests that pre-treatment for a limited period in high concentrations of penicillin has little additional effect and that the inhibition he observed is explicable in terms of the bacteriostatic effect of the penicillin present during his sterility tests.

The results with the higher penicillin concentrations offered some hope that the prolonged bacteriostatic effect would prevent survival of the bacterial flora without adversely affecting the diatoms. Samples of a vigorously growing culture of *Nitzschia closterium* forma *minutissima* were dispensed in plugged sterile tubes and amounts of penicillin added to give resultant concentrations of 50, 100, 500, 1000, 5000, and 10,000 units/ml. The cultures were kept in a north window. In those containing 5000 and 10,000 units/ml. diatom growth was obviously inhibited, the cells settling and finally loosing all colour The diatoms in the lower concentrations grew well and showed no obvious difference from a control culture and were, after 16 days, still viable on sub-culturing to penicillin-free media. At the same time as this diatom viability test, one ml. samples of all tubes showing diatom growth were inoculated into (*a*) peptone sea water, and (*b*) peptone sea water agar. The results of this test re shown in Table II and indicate the essentially bacteriostatic action of the enicillin. Thus, though 100 units penicillin per ml. is sufficient to inhibit all rowth in peptone sea water for over 21 days, some bacterial cells are still iable and will grow on subculture to penicillin-free peptone sea water. The egative results obtained in peptone sea water on inoculation with samples rom the diatom culture which had contained 1000 units/ml. might be due to ufficient residual antibiotic activity having been carried over with the noculum. On the other hand, on reinoculation of this tube some growth was btained. Moreover, these penicillin concentrations yielded positive results vhen samples were used to inoculate peptone sea water agar. The same

ABLE II. Effect of Penicillin on the Survival of the Bacterial lora Associated with a Growing Culture of *Nitzschia closterium*

Penicillin concentration in diatom culture (units/ml.)	Bacterial growth	
	P.S.W.	P.S.W.A.
50	+	+ (Heavy)
100	+	+ (Heavy)
500	+	+ (Slight)
1000	–	+ (Slight)

1 ml. quantities of a diatom culture which had grown for 16 days in the presence of stated mounts of penicillin inoculated into (*a*) peptone sea water (P.S.W.), and (*b*) peptone sea water gar (P.S.W.A.).

ilution factor was involved in this case and it seems most probable that the rolonged bacteriostatic effect of the penicillin is sufficient to prevent survival f the majority of the bacterial flora which will grow easily in peptone sea vater. Some cells of the flora survive and continue to grow on transference o a penicillin free media. These 'resistant cells' seem to be culturally different rom the bulk of the flora in that they will not easily grow in peptone sea water ut do so in an identical medium solidified with agar.

A similar series of experiments with streptomycin showed that this antiiotic was not as effective a bacteriostatic agent for the bacterial flora ssociated with a diatom culture as penicillin (see Tables III, IV and Fig. 1). t was, moreover, more inhibitory to diatom growth, 500 units/ml. producing marked inhibition. A comparison of the gross cultural characteristics of the streptomycin resistant' bacteria on peptone sea water agar showed a marked redominance of orange- or brown-pigmented colonies. In contrast, these rganisms were sparsely distributed amongst the colonies that survived rolonged penicillin exposure. This suggested that the two antibiotics might e active against different sections of the bacterial flora and the possibility of he use of mixtures of the two was therefore investigated. A vigorously rowing culture of *Nitzschia closterium* forma *minutissima* was dispensed in

Table III. Effect of Streptomycin on the Growth in Peptone Sea Water of the Bacterial Flora Associated with a Diatom Culture

Concentration of streptomycin (units/ml.)	Days incubation before growth first visible (turbidity)
Nil	2
5	4
10	4
50	5
100	7
500	10
1000	11

To tubes of peptone sea water were added various concentrations of streptomycin. Tubes inoculated with 1 ml. of a crude culture of *Nitzschia closterium*. Observed daily and day when growth first seen in each tube (turbidity) noted.

Table IV. Effect of Streptomycin on the Survival of the Bacterial Flora Associated with a Growing Culture of *Nitzschia closterium*

Streptomycin concentration in diatom culture (units/ml.)	Bacterial growth	
	P.S.W.	P.S.W.A.
50	+	+
100	+	+
500	+	+
1000	+	+

1 ml. quantities of the diatom culture which had grown for 16 days in the presence of stated amounts of streptomycin inoculated into (*a*) peptone sea water (P.S.W.), and (*b*) peptone sea water agar (P.S.W.A.).

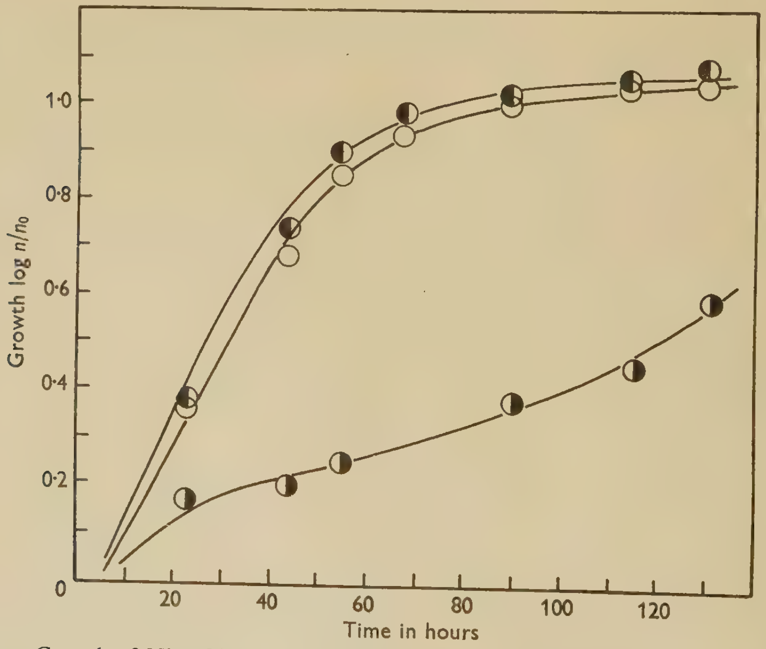

Fig. 1. Growth of *Nitzschia* in enriched sea water (for details see text) with additions of antibiotics. Growth plotted as log n/n_0, where n_0 = number of cells per ml. at zero time and n = the number at time t. —○—○—, control (no antibiotic added); —◐—◐—, 500 units/ml. penicillin; —◑—◑—, 500 units/ml. streptomycin.

plugged sterile tubes and penicillin and streptomycin added to give resultant concentrations of 50, 100 and 500 units/ml. of both. The tubes were illuminated in a north window for 16 days during which time diatom growth occurred (somewhat inhibited in the highest concentration), all concentrations finally yielding viable diatom cells on subculture to antibiotic-free medium. At the same time 1 ml. quantities of the culture were tested for bacterial growth by inoculation into (*a*) peptone sea water, and (*b*) peptone sea water agar. The results are shown in Table V.

TABLE V. EFFECT OF PENICILLIN AND STREPTOMYCIN ON THE SURVIVAL OF THE BACTERIAL FLORA ASSOCIATED WITH A GROWING CULTURE OF *NITZSCHIA CLOSTERIUM*

Antibiotic concentration (units/ml. of both)	Bacterial growth	
	P.S.W.	P.S.W.A.
50	+	+ (Slight)
100	+	–
500	–	–

1 ml. quantities of a diatom culture which had grown in the presence of stated amounts of both penicillin and streptomycin for 16 days inoculated into (*a*) peptone sea water (P.S.W.), and (*b*) peptone sea water agar (P.S.W.A.).

The diatom cells grown in the presence of 500 units/ml. of both streptomycin and penicillin showed no bacterial growth after inoculation into both testing media. On reinoculation of these tests with 1 ml. of a crude diatom culture, only slight growth was obtained in peptone sea water and none on peptone sea water agar, and it therefore seems probable that the negative results obtained were due to residual antibiotic activity being carried over with the inoculum. Considerable numbers of diatom cells were still viable and good growth was obtained on inoculation into sterile enriched sea water without additions of the antibiotics. On repeating the same procedure with other samples of the same crude diatom culture, treatment with 500 units/ml. of the two antibiotics did not always yield completely negative sterility tests of the type shown in Table V. Regardless of this, the bacteriostatic effect of the antibiotics is always very considerable and providing a vigorous diatom growth has occurred the ratio of bacteria : diatoms will always have increased tremendously in favour of the diatoms. It has invariably been found possible to obtain a bacteria-free subculture of the diatom by inoculating numerous samples of sterile enriched sea water with very small inocula from the antibiotic treated culture (e.g. loop inocula of approximate volume 0·006 ml.). 1 ml. samples from a proportion of such subcultures normally prove to be bacteria-free. One such strain of *Nitzschia* has been maintained for several months by serial subculture in sterile medium. Regular sterility tests show it to be still bacteria-free.

A preliminary investigation has been made of the possibility of applying the technique to other phytoplankton cultures. Of numerous organisms tested only one Chrysophyceae (*Chromulina pleiades* Parke), one Dinophyceae (*Peridinium trochoideum* (Stein) Lemm.) and three Chlorophyceae (*Chlorella* sp., *Chlamydomonas* sp., and *Stichococcus* sp.) would grow in the presence of 500 units/ml. of penicillin and streptomycin. By dropping the streptomycin concentration to 100 units/ml. the growth of two other Chrysophyceae (*Isochrysis galbiana* Parke, and Flagellate 25 Plymouth), *Prorocentrum micans* Ehrenb. and *Coccolithus huxleyi* (Lohm.) Kamptner occurred. No members of the Cryptophyceae tested showed any growth except *Hemiselmis rufescens* Parke, which gave variable results. A feature of these latter investigations was the reluctance to grow on subculture from the antibiotic-treated culture to ordinary Erdschreiber of those members of the Dinophyceae and Chrysophyceae which had produced good growth in the presence of the antibiotics.

DISCUSSION

The preliminary investigations reported in this note indicate that the use of penicillin and streptomycin for obtaining bacteria-free cultures of diatoms depends upon their selective bacteriostatic action. It seems possible that the high concentrations used do exert some bactericidal effect, but some of the bacterial flora survives long exposure under these conditions. Success, therefore, depends upon some form of extinction dilution procedure, though under suitable conditions this can be reduced to the use of small inocula for subculture from the antibiotic-treated media.

It must be emphasized that sterility tests can give misleading results if there is a chance of sufficient antibiotic activity being carried over with the inocula, and in view of this such tests are best carried out on samples of subsequent subcultures in sterile antibiotic-free medium. In any case it is desirable that several successive subcultures in the absence of antibiotics should prove to be sterile before the culture is considered to be bacteria free. In addition, experience has shown the desirability of extensive sterility testing on a variety of media. Cultures have frequently been obtained which yielded no bacterial growth on peptone sea water agar but did in peptone sea water and vice versa. The casein medium has sometimes shown the presence of bacteria (and actinomycetes) which did not grow on either peptone sea water or peptone sea water agar. The latter medium (Zobell, 1941) seems, however, to satisfy the requirements of most marine heterotrophes and the use of both liquid and solid media for sterility testing appears more important than the use of media of widely differing nutrient composition.

The antibiotics used are not effective against moulds and actinomycetes and their addition in the quantities used causes an enhanced growth of such organisms. It is essential that the crude phytoplankton cultures used should be free of such organisms, otherwise the culture will be heavily contaminated vith mycelial growth. Vigorously growing algal cultures are, however, ıormally only slightly contaminated with such organisms and a mould-free lgal culture can normally be obtained by the usual washing procedure prior o antibiotic treatment. It is of interest that very heavy mycelial growth which ccurred in some flagellate cultures treated with antibiotics did not cause any narked inhibition of algal growth.

Throughout these studies it has been obvious that organisms which best urvived the antibiotic treatment were those of a robust nature (e.g. *Nitzschia* nd members of the Chlorophyceae). In general these organisms are known o be non-exacting in their nutritional requirements and will grow in Miquel Sea Water. Organisms which need soil extract for continued growth are the nost affected by the antibiotics. The organisms of this class, which will olerate the action of the antibiotics, frequently do not grow on subsequent ubculture to sterile antibiotic-free media. In such it is possible that the ntibiotics have directly affected the algae. It seems more probable, however, hat such effects are due to an indirect action. If the more nutritionally xacting algae are dependent upon symbiotic bacteria for essential nutrients r growth factors, then control of the bacterial flora may ultimately limit lgal growth. Such considerations make the direct application of the echnique to a wide variety of phytoplankton organisms difficult. For any rganisms with complex nutritional requirements, media other than Erd-chreiber may have to be used in the absence of the symbiotic bacteria.

SUMMARY

o develop a technique for the isolation of bacteria-free algal cultures, the se of penicillin and streptomycin for controlling bacterial growth has been nvestigated.

By using the selective bacteriostatic properties of the antibiotics it has been ound possible to increase the ratio algal cells : bacteria markedly in favour of he algae. From suitably treated cultures it has been found possible to obtain ubcultures of *Nitzschia closterium* forma *minutissima* and two members of the hlorophyceae which produce no bacterial growth on a variety of media.

A preliminary investigation of the application of the technique to other lgal cultures has been made. The possibilities and difficulties of this are iscussed.

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UNDERWATER OBSERVATIONS ON THE SWIMMING OF MARINE ZOOPLANKTON

By Richard Bainbridge

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(Text-figs. 1–4)

Many experiments have been made in the laboratory by different workers to study the swimming behaviour of plankton animals in relation to light and other factors. At the present time the special apparatus described by Hardy & Bainbridge (1951) is being used for a study of vertical migration, and other experimental work on the inter-relationships of zooplankton and phytoplankton (Bainbridge, 1949) is also in progress. Both this type of work and current theories (see Cushing, 1951) on the migrations of zooplankton make it of some consequence to learn the actual nature of the movements of these animals in the sea itself; and it is of special importance to compare their swimming behaviour in the different types of apparatus with their behaviour in the sea.

Dammant (1921), whilst hanging below a ship during salvage operations, had already made interesting observations on mackerel feeding upon members of the plankton and shown that a diver could see quite small plankton animals under natural conditions; and Beebe (1935), making descents in the bathysphere, had frequently remarked on the visibility of plankton, although his primary aim was the observation of larger forms such as fish.

Simple diving helmets as commonly used in warmer seas are not suitable for sustained observations in colder waters: a more protective and elaborate diving suit is required. Professor Hardy, with whom I was working under a Leverhulme Research Grant, consequently approached Messrs Siebe, Gorman and Company, and their assistance was most generously and freely given. They provided training in the use of various types of diving gear and made a loan for the greater part of the summer of that type of gear which was finally decided to be most convenient for the work.

I am deeply indebted to Messrs Siebe, Gorman and Co., whose provision of training and loan of equipment made the work possible; to Professor A. C. Hardy, who gave every encouragement and who made the time and facilities available; and to Muriel C. Morris, and the staff and boat crews of the Millport Marine Station upon whom my safety depended.

EQUIPMENT AND METHODS

The gear chosen was a modified compressed-air breathing apparatus together with a two-piece frog suit. The breathing apparatus had the normal face piece and a demand valve on the harness, but had a rubber air hose connected to a reducer valve on large (165 cu.ft.) compressed-air cylinders on the shore. The hose could be employed as a life-line and the standard cylinders were of greater capacity and were more easily replaced when used than the small cylinders usually carried on the diver's back. The hose allowed of quite sufficient movement for the type of observing desired and, with practice, swimming with foot flippers was possible, while more weights and the use of heavy boots allowed for walking on the bottom.

The work was performed at the Millport Marine Station and tours of observation of three kinds were tried. First, the observer simply walked down the shore to a suitable depth. This involved moving amongst rocks and in the seaweed zone and stirring up a good deal of detritus, with the result that viewing was sometimes made rather difficult. The site chosen for this method was sheltered and still. Secondly, the observer went down a ladder from Keppel Pier directly into deeper water with a hard shingly bottom. The water here was generally clearer but was without tidal movement only for very limited times during the day, whereas at the first site it was still for much longer. Thirdly, he was lowered to a suitable depth in a bosun's chair over the side of the research ship '*Calanus*', which was either anchored or drifting over much deeper water. All these methods could be attempted only when weather and tidal conditions were exactly right and this naturally greatly limited the scope of the work. A fairly clear sky, with sun if possible, little wind and slack water were found to be most convenient. Thick woollen clothing allowed tours of up to 40 min. duration in water of 6·5° C. without very great discomfort.

Under suitable conditions it was found easy to see and recognize many copepods. *Calanus finmarchicus* was visible up to 4 or 5 ft. away in clear water, while amongst the smaller forms those more opaque, such as *Temora* and *Centropages* spp., were visible up to a foot away or even more. The clearest views could be seen by looking about 10° either to the left or to the right of the bright patch formed by the sun on the surface of the water. In this manner a sort of dark ground illumination is obtained and even the most transparent forms stand out very clearly. By crouching or standing it is possible to follow vertical movements of about 10 ft. in extent while with the flippers greater distances are feasible, although the observer's movement is then more erratic and actual distances less easy to judge with accuracy. Chains of diatoms such as *Thalassiosira* and *Skeletonema* spp. and even individuals of larger species, such as *Coscinodiscus*, were frequently visible as occasionally were such flagellates as *Noctiluca* and *Phaeocystis*. Need for sufficient sunlight naturally limits good viewing to the more surface waters and observations were not generally made at a greater depth than 20 ft. Altogether twelve descents were made over a period of about 5 weeks.

OBSERVATIONS

In the Clyde Sea area the spring brood of *Calanus* is often to be found in large numbers in the surface layers throughout the 24 hr. and is therefore particularly suitable for investigation. It does not, of course, follow that the behaviour of this brood, under these conditions of lighting, represents the normal behaviour of the copepod which is at other times of the year found in much deeper layers (at least 50 m. down); but it may be allowed as an indication of the probable type of behaviour at these greater depths, where observation is not yet possible.

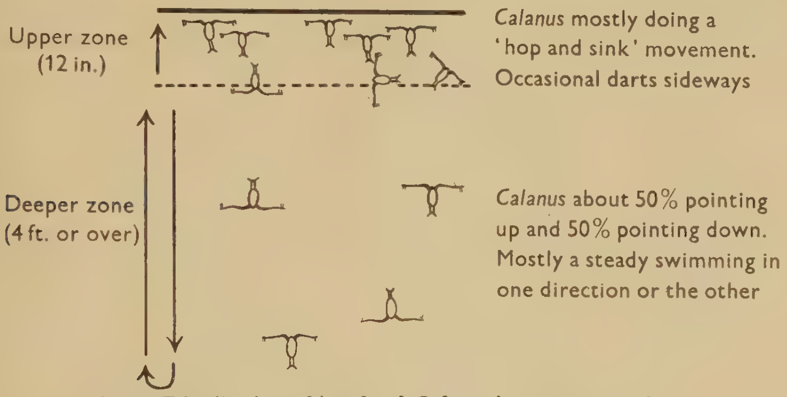
Upper zone
(12 in.)
Calanus mostly doing a 'hop and sink' movement. Occasional darts sideways
Deeper zone
(4 ft. or over)
Calanus about 50% pointing up and 50% pointing down. Mostly a steady swimming in one direction or the other

Fig. 1. Distribution of 'surface' *Calanus* in upper water layers.

Two zones of differing behaviour are clearly recognizable in the spring *Calanus* (Fig. 1). In the upper 12 in. of water there is a high concentration of animals (up to as many as 10/sq.ft. of surface) and there is a continuous gentle sinking and swimming up again vertically within this zone. Occasionally violent oblique or horizontal darts of several feet may occur, especially when two or more animals come close to each other; and a good deal of horizontal movement may result from a sort of bouncing on the underside of the surface film. The *Calanus* in this zone are often aggregated into groups of a dozen or so which sometimes swim round and round each other like a group of mayflies. Both in these little swarms and on a larger scale, like most other plankton animals, they are extremely patchy in distribution. Frequently, after the tedious and complex preparations for diving, one finds that there is no zooplankton in the area at all and on other occasions there may be relatively few swarms, or shoals, with large stretches of empty water between them. This patchiness was amply confirmed by the variable results of hauls with plankton nets.

Below the upper zone of 12 in. is a second one of indeterminate depth. Animals are scarcer in this zone and are more uniformly distributed. They appear in about equal numbers swimming either vertically upwards or downwards and seem to be going to or from the upper layer. I was able to follow (separately) two or three individuals which left the upper layer, turning over and swimming vertically and steadily downwards for 5 or 6 ft., where they again turned upright and hung motionless for a short time before swimming steadily up again to the surface layer. Others swam up and down, presumably to and from greater depths, but I was only able to follow the few individuals making more shallow excursions. It may be that this deeper zone is one of migration between two more concentrated zones, the one at the surface and the other either just above the bottom or at some optimum level about 50 to 60 m. down. Tow-nettings on these occasions certainly show animals in large numbers both at the surface and much deeper. It is reasonable to suppose that the whole population may be uniform and that there is a constant interchange between what are generally termed the 'surface' and 'deep' forms.

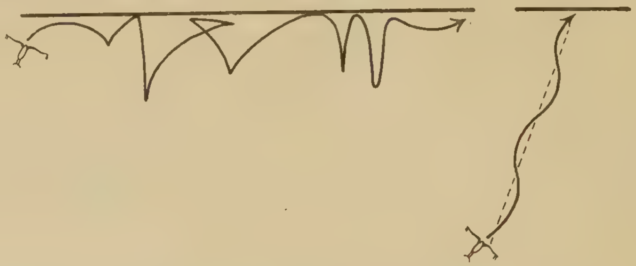

Fig. 2. Two types of swimming movement by *Calanus*.

An outstanding feature of all the populations observed was that, at any one time, as high a proportion as 50% would be quite motionless, most of these remaining so for long periods and many so delicately balanced as not to be even sinking in the water. On some occasions, especially when the sky was overcast, the whole population would be hanging motionless or drifting passively.

A clear horizontal migration by *Calanus* was seen only once. This occurred when there were very few specimens in view and the two seen, in the words of my diary, 'were just under the surface film and were moving horizontally by a series of oblique leaps and looping movements. They did not go deeper than about six inches. Horizontal progress was quite rapid and there were only very short motionless pauses' (Fig. 2). On the same occasion I watched another *Calanus* swim up about 3 ft., to the surface, in a rapid spiralling movement at an angle of about 30° to the vertical.

Observations on animals other than *Calanus* were only incidental, but the following points may be of interest.

Many smaller copepods were frequently seen in the upper 2 or 3 in. of ater. These seemed to swim in an entirely random fashion with very little rtical and a good deal of horizontal movement.

Sagitta was common, generally exhibiting a very rapid vibration of the hole body, but showing little forward movement. I was able to make a cord, on a piece of ground perspex, of the orientation of a specimen at 5 sec. tervals. This is reproduced in Fig. 3 and shows that, over a period of 1 min., e anterior end was generally pointing upwards but rarely vertically so.

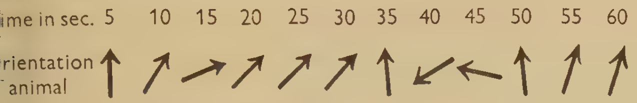

Fig. 3. Orientation of *Sagitta* sp. at 5 sec. intervals.

Coelenterates and ctenophores were almost always resent and their swimming movements and the effect n these of such crude stimuli as touch were easily bserved. A small gadoid associated with a medusa robably *Cyanea*) was watched for some time and the sh was seen generally above the bell in an almost ertical position. It repeatedly sank down towards the ell and then swam up again (Fig. 4). The coelenterate as gently pulsating and the pair gradually drifted bliquely upwards. The fish showed no alarm and made o attempt to conceal itself under the bell during bservation.

On another occasion large shoals of saith about 2 in. ng were watched. The most striking feature about em was the constant very vigorous movement of the hole body. They again appeared undisturbed by bservation unless I waved my arms in the midst of a oal.

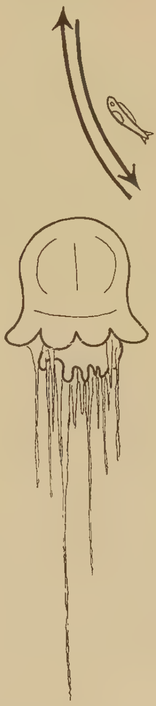

Fig. 4. Detail of swimming movement of small fish associated with a coelenterate.

Discussion

can be said with fair confidence that few of the animals observed were isturbed in any apparent way by the presence of the diver. Those which did ow a reaction to his presence were the larger bottom-living fish and rustacea. The type of breathing set chosen shares, with all but those utilizing xygen and absorbent salts, the disadvantage of releasing used air into the ater. This, however, escapes behind the diver and only when he is breathing ut. It is quite simple to regulate breathing so that, during crucial minutes, ere is no air escaping. When it does so it causes no noticeable movement of the water and animals do not appear to react to it. The observations on th planktonic forms are therefore believed to be valid as records of their natura behaviour under the general environmental conditions mentioned. *Calan* in particular showed a great preponderance of vertical movements in it swimming and it seems reasonable to deduce from this that the diurna vertical migrations that it performs at other times of the year are a direct an straight swimming up or down through the water column, and not, as ha been suggested by some authors, the result of random movements. A rando type of swimming is, however, sometimes indulged in, particularly when nea the surface film and when the population density is high. Those smalle copepods which are more often permanently at the surface seem more pron to swim in a random fashion. This behaviour may be a function of their ver high population density.

However scientific and detached an account of the movement of thes animals may be, it would be wrong not to make mention of the extraordinar beauty both of these forms and of their underwater surroundings. Experienc of its richness and its strangeness would in itself be more than adequat reward for the discomfort and effort of diving.

SUMMARY

Attempts to make underwater observations on the movement of plankto animals using a modified frogman apparatus are described.

With practice, observation of even the smallest planktonic animals, unde suitable conditions, is perfectly feasible.

Most data were obtained about individuals of the spring brood of *Calan* inhabiting the surface layers. This population occupied two zones—an uppe 12 in. where movement was generally a ‘hop and sink’ one and occasionall random, and a deeper zone where individuals were found swimming in equa numbers vertically upwards and downwards.

Calanus is often to be found in small swarms or clusters of a dozen or s individuals.

Smaller copepods and younger stages in the upper layers frequently indulg in a random motion.

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STUDIES ON *CHAETOPTERUS VARIOPEDATUS* (RENIER). III. FACTORS AFFECTING THE LIGHT RESPONSE

By J. A. Colin Nicol

Zoologist at the Plymouth Laboratory

(Text-figs. 1–26)

Luminescence in *Chaetopterus* is an extracellular phenomenon and is due to the secretion of photogenic material by certain glandular cells in definitely circumscribed regions of the body. These gland cells are eosinophilic elements scattered singly or massed together in dense aggregations in certain regions. They are particularly abundant in the epithelium covering the distal surface of posterior notopodia, and in two glandular areas on the dorsal surface of the aliform notopodia. These two regions also display the brightest luminescence (Nicol, 1952*a*).

The gland cells producing the photogenic material are under nervous control in *Chaetopterus*, and secrete only as the result of stimulation. A preliminary investigation of light production in this animal has established certain facts about the nature of nervous regulation (Nicol, 1952*b*). With electrical stimulation it has been found that a single shock, above threshold strength, will induce secretion and luminescence, and that the amount of secretion, and hence luminescence, is augmented by increasing the number of stimuli. The luminescent powers of the animal are soon fatigued or exhausted, however, under repetitive stimulation, and after a few shocks the light tends to diminish. Moreover, it was also noticed that at low frequencies of stimulation the light response was confined to the region directly stimulated, but as the frequency was raised the response showed a tendency to spread to other parts of the body. This effect was most pronounced in the posterior region of *Chaetopterus*.

The present study is concerned with analysing in greater detail the factors concerned with regulation of the magnitude of the luminescent response. In particular, attention has been concentrated on the effect of altering the frequency and number of impulses. The regions found most favourable for study were the aliform notopodial light glands and the notopodia of the posterior region, and quantitative investigations were confined to these structures.

I am indebted to Dr W. R. G. Atkins, F.R.S., for the loan of a galvanometer nd camera used in this investigation, and to Mr F. J. Warren for technical ssistance. Part of the expenses incurred in this research was defrayed by grant-in-aid of scientific investigations from the Royal Society.

MATERIAL AND METHODS

The methods of recording luminescence have already been described (Nicol, 1952*b*), and involve the employment of a multiplier photocell, and a sensitive galvanometer or oscilloscope. More use of the latter instrument was made in the present investigation. Electrical stimuli consisted of condenser discharges from an electronic stimulator, unless otherwise stated, and were delivered through platinum electrodes laid on the surface of the animal.

OBSERVATIONS

Luminescence in the Aliform Notopodia

The experiments described in this section consisted of stimulating the light glands on the aliform notopodia, and of recording the light which resulted. The effects of altering the frequency and number of stimuli were explored.

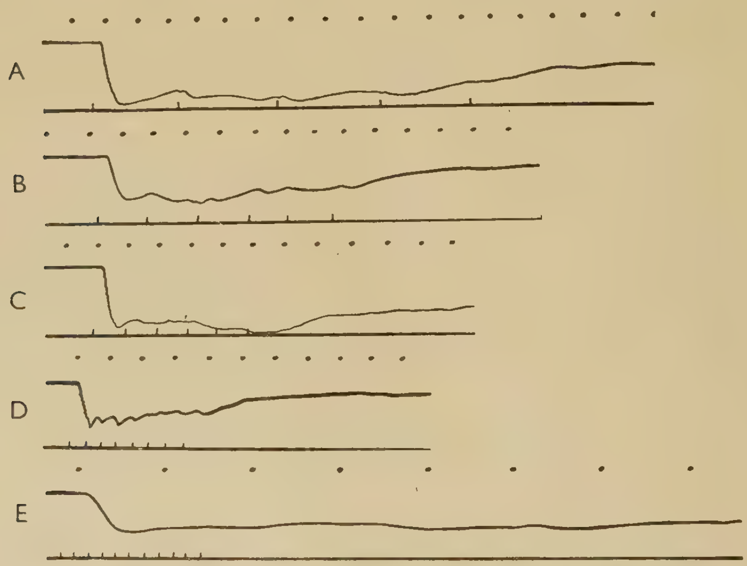


Fig. 1. Stimulation of the light glands on the aliform notopodia at different frequencies. Each record was obtained from a different specimen. A, stimulation 1 per 30 sec.; B, 1 per 15 sec.; C, 1 per 10 sec.; D, 1 per 5 sec.; E, 3 per 5 sec. Time scale above each record, 1 per 10 sec. Stimuli shown on bottom lines. In A–D separate inflexions can be seen, in relation to the separate stimuli. In E, however, the response curve becomes smoothed out. Oscilloscope records.

Following a suggestion made by Professor A. V. Hill, F.R.S., I have prepared a series of records to permit a comparison of the general character of the luminescent response with records of muscular contraction (Fig. 1). Stimuli were delivered at low frequencies of 2–36 per min., and the responses were photographed at low camera speeds. At the lower frequencies (Fig. 1 A–D) definite inflexion can be seen corresponding to each stimulus. These nflexions result from the discharge (secretion) of photogenic material. Above rate of 30 per min. the separate inflexions tend to become smoothed out Fig. 1 E) and a response curve somewhat resembling a curve of tetanic contraction is produced. At the low frequencies employed in this series of experiments he response to the first stimulus is maximal, and subsequent responses do not xceed the initial response. The rate of decay of luminescence, however, is ery slow, and successive stimuli tend to maintain the initial level of light ntensity. The reduced response to the second and succeeding stimuli is due o some kind of fatigue, and it is not possible to demonstrate summation at hese low frequencies (below 1 per sec.).

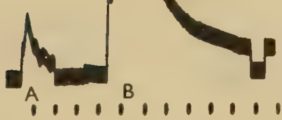
In a previous communication (Nicol, 1952*b*) some observations were resented to show that by increasing the number of stimuli the height of the esultant light response was increased in consequence. This effect has been xplored further to determine the nature of the summation process involved nd the relative roles of frequency and number of impulses in determining he magnitude of the response. A very extensive series of experiments was arried out on the light glands of the aliform notopodia, and over 150 animals ere used.

In order to have comparable records showing the differential effects of arying the experimental conditions, say the frequency of stimulation, it is ecessary to be able to make repeated observations on the same animal. atiguing of the light response is very marked in *Chaetopterus*, however, and ecovery has not been observed in any period of time that would be advantageous for experimentation. The experiments to be described consisted of timulating an animal in a given way, allowing a rest period of 5 min. in hich decay of luminescence could occur, and then stimulating once more at higher rate or for a longer period. Periods of stimulation were kept as brief the experiment would allow. These experiments have been carried out on he assumption that the response to a second stimulation, which duplicates he first stimulation, may be equal to the first response or less than the first esponse, but will not be greater. Furthermore, if fatigue should occur, it is ossible that any augmented response, resulting from increasing some haracteristics of the stimulation, may be sufficient to offset the fatigue effect, nd reveal itself as an increment over the first response.

In general, it has been found that when a specimen is subjected to successive periods of identical stimulation, and the conditions of the experiment re maintained constant, the succeeding responses fall below the level of the nitial response.

Effect of Increasing the Frequency of Stimulation

To determine any possible effect dependent upon frequency of stimulation, pecimens were excited electrically for two periods, once at a low frequency, and again after a period of rest, at a higher frequency. To eliminate th influence of varying the number of impulses, the duration of the stimulatio period was regulated so that the same number of impulses (within 10%) wa administered on each occasion. Impulse duration and voltage were kep constant. Frequencies which were used ranged from 1 to 33 per sec., and th number of stimuli varied from three to twenty-one in the different experiments



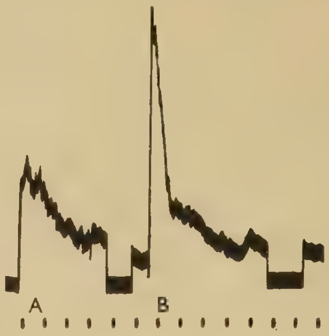


Fig. 2 (*left*). Stimulation of the light glands on the aliform notopodia. A, response t a single electrical stimulus; B, twenty-nine stimuli at 1 per sec.

Fig. 3 (*right*). A record showing the effect of stimulating the light glands at differen frequencies. A, 4½ sec. burst at 2 per sec.; B, 1 sec. burst at 9 per sec. Time scale belo both figures, 1 per min.

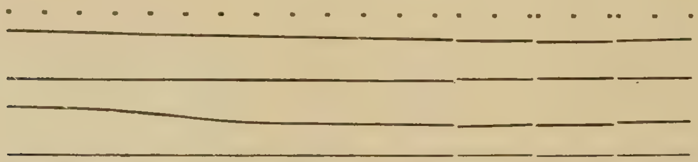


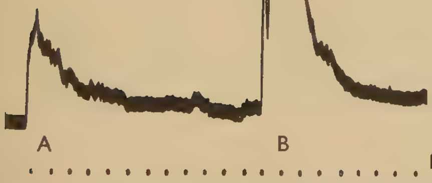
Fig. 4. Oscilloscope records of light produced by stimulation of the light glands on th aliform notopodia at different frequencies. Top record, 2 sec. burst at 5·5 per sec. Botto record, 0·5 sec. burst at 23·4 per sec. Time scale, 1 per 2 sec. Interruptions in the recor are intervals of 20 sec.

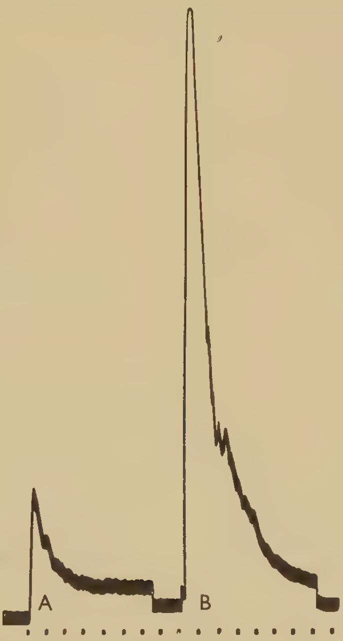
A number of definite and clear-cut positive results was obtained in whic the second burst of stimuli, at a higher frequency, resulted in a much large response than that evoked by the previous stimulation at a lower rate. Th increment lay both in the initial response peak, and in the total amount o light produced. A typical record, obtained by photographing a galvanomete deflexion, is shown in Fig. 3. In this experiment the first stimulation con sisted of a 4·5 sec. burst at 2 per sec., the second stimulation of a 1 sec. burs at 9 per sec. The peak intensity of light resulting from the higher frequenc is about twice as great as that evoked by the lower frequency, but the tot light is about the same in both. In other records initial and total light showe a pronounced increase (Figs. 4, 5).

Effect of Increasing the Number of Stimuli

By increasing the number of stimuli it is possible to increase the amount of ght produced, but the amplitude of the effect varies with the specimen. An gmentation of the light response on increasing the number of stimuli first ecomes apparent at a frequency of about one per sec. (Fig. 2).

Increasing the number of stimuli has the same kind of augmentative effect raising the frequency, but the effect is more pronounced and is more easily icited. The height of the response, that is the initial intensity, and the total ght emitted, that is the amount of secretion, are both increased by prolonging e duration of stimulation (Fig. 7).





g. 5 (*left*). Galvanometer records of light produced by glands of the aliform notopodia. A, twenty shocks at 5·5 per sec. for 3·6 sec.; B, nineteen shocks at 32·2 per sec. for 0·6 sec.

g. 6 (*right*). A record of light produced by glands of the aliform notopodia after dissection and removal of the nerve cord. A, a burst of eighteen stimuli at 1·3 per sec. for 14 sec.; B, eighteen stimuli at 9 per sec. for 2 sec.

The upper half of the second response curve in both records (B) has been extrapolated on the basis of visual readings of galvanometer deflexions. Time scale, 1 per min.

It is possible to consider two mechanisms that could be operating in these sponses, one a simple effect of summation in the effector, and the other facilitatory effect induced by the build-up of an excitatory state under olonged rapid stimulation. Facilitation, if present, could be peripheral or ntral, since the preparation consisted of the whole animal. The data pre-nted above are not adequate to allow a choice among these alternatives, and towards this end the experiments described in the following four sections were carried out. These experiments consisted of testing the effects of various anaesthetics on the luminescent response; stimulating the nerve cord in contradistinction to the peripheral field; stimulating pieces of glandular tissue which were severed from the nerve cord; and comparing the effects of one versus two electrical stimuli. The results may be anticipated by stating that they favour the concept of a peripheral contractile mechanism controlling secretion, and capable of summation under repeated stimulation.

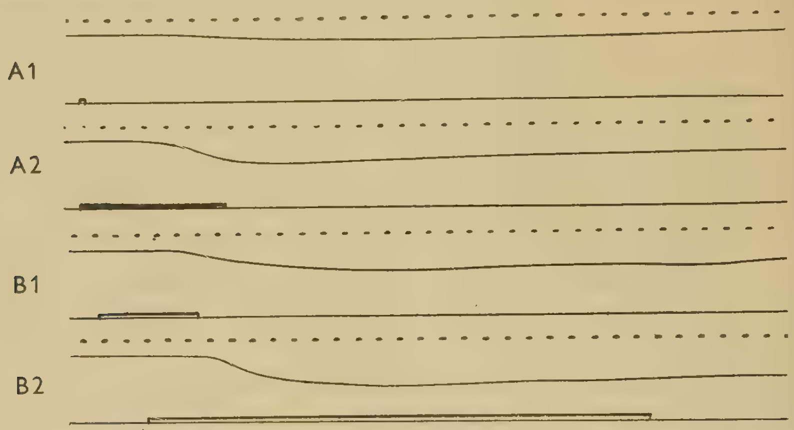


Fig. 7. Effect of number of impulses on light production in *Chaetopterus*. Oscilloscop records of luminescence in the aliform notopodia. A 1 and A 2 from the same animal stimulated at a frequency of 25 per sec. A 1, burst of 0·2 sec. duration; A 2, burst of 5 sec duration. B 1 and B 2 from another animal, stimulated at 2·2 per sec. B 1, duration 4 sec. B 2, duration 22 sec. Stimulation represented by horizontal lines at bottom of records time scale above each record 1 per sec.

Effects of Anaesthetics on the Luminescent Response

Several anaesthetics, that are known blocking agents of nervous tissue, wer employed in an attempt to prevent nervous transmission without halting th activity of the peripheral effector concerned with the luminescent respons The anaesthetics employed were chloretone (acetone chloroform), MS. 22 (tricaine methansulphonate: Rothlin, 1932), cocaine, stovaine, procain eucaine, ether, and isotonic magnesium chloride ($MgCl_2.6H_2O$, 7·3 %).

Stimulation of the Anaesthetized Gland Cells

In the following experiments the animals were anaesthetized for varyin periods of time, and the light glands were then stimulated directly by placin a pair of platinum electrodes on them. The animals were then placed i running sea water for several hours to wash out the anaesthetic, followin which they were stimulated as before. Stimulation consisted of 10 or 20 se bursts at 5 per sec.

Chloretone (0·1 %). Specimens were immersed in this anaesthetic for –30 min. After 10 min. the anaesthetic caused a marked diminution in the mount of light produced, which was only about a third of that given off by he same animal free of anaesthetic (Fig. 8).

MS. 222. Animals immersed in this anaesthetic for 10–30 min. showed reatly reduced luminescence under electrical stimulation when compared ith the same individuals free of anaesthetic (Fig. 9). The results were similar o those obtained with chloretone.

Stovaine (0·5 %) and *eucaine* (0·5 %). These two drugs were tried at several oncentrations and for various periods to determine effective dosages and ction times. Concentrations of 0·5 % acting for 15 or 30 min. proved ffective in greatly reducing luminescence while still permitting subsequent ecovery (Figs. 10, 11). A peculiarity of many of the records obtained is the ccurrence of a small primary peak before the luminescence reaches its aximum in specimens from which the anaesthetic has been washed out.[1]

Cocaine. Solutions of cocaine hydrochloride were used at concentrations f 0·5 % (pH 7·6) and 1 % (pH 7·3). After acting for 30–60 min., these olutions greatly reduced the luminescence produced by electrical stimulation Fig. 12).

Procaine at concentrations 0·5–2 %, acting up to 30 min., failed to signicantly reduce the amount of luminescence. Solutions of 4 % were then ade up, and the pH was returned to 8·0 with NaOH. Procaine, at this ncentration, greatly reduced luminescence in 30 min. (Fig. 13).

Ether (0·1 %) failed to significantly reduce luminescence in 1 hr.

Isotonic *magnesium chloride* (pH 8·2). After immersion for 15–30 min. in is solution the animals showed greatly reduced luminescence to electrical imulation (Fig. 14).

The noteworthy feature of all these results (except with ether) is that the naesthetic greatly diminishes but fails to completely abolish the luminescent esponse. The drugs greatly reduce the intensity of light, and appear to estrict its appearance to a narrowly confined region immediately underneath he electrodes. The responses under anaesthesia are interpreted as suggesting hat nervous transmission has been blocked, but that the luminescent effector s still responding directly to electrical stimulation.

timulation of the Nerve Cord in Anaesthetized Animals

Evidence for this viewpoint has been sought by stimulating the nerve cord irectly. Specimens were anaesthetized as before, using MS. 222 for 30 min. hey were then pinned out on a platform with ventral side uppermost and ith the dorsal light glands in the aliform notopodia exposed through an perture situated above a mirror. Fine silver electrodes arranged to give

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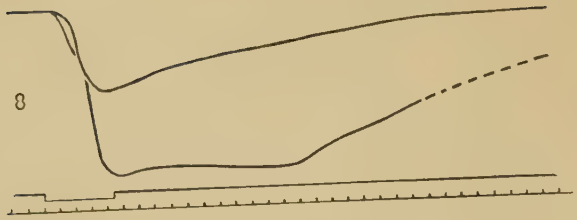
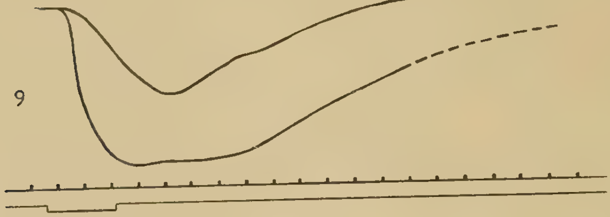
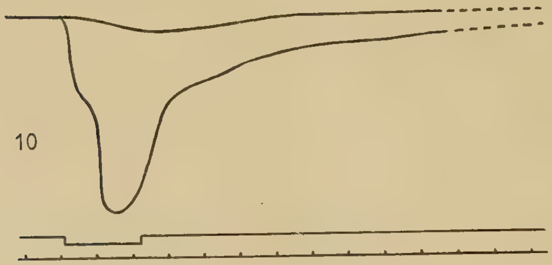
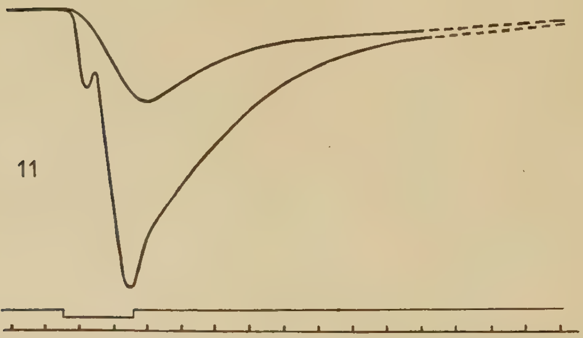
[1] This effect, also seen in other records, is due to quantitative differences in the secretion roduced by the two luminescent glands of the aliform notopodia.



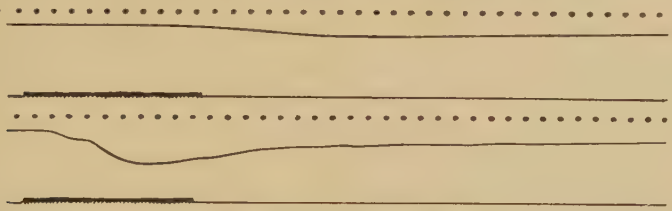




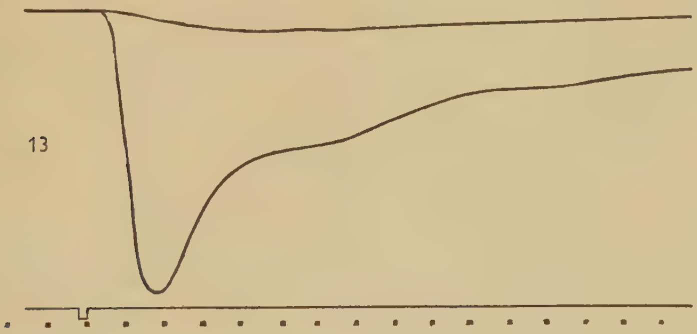


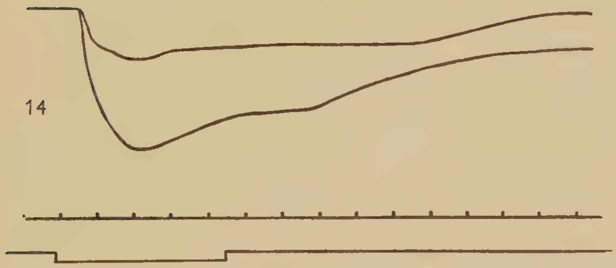
Figs. 8–11. Luminescent responses of specimens when anaesthetized, and after recover from anaesthesia. The upper curve in each record is the response under anaesthesi the lower curve, the response after washing out the anaesthetic. Stimulation, indicate as a horizontal interval, consisted of a 10 sec. burst at 5 per sec. Fig. 8, chloretone 0·1 for 10 min. Fig. 9, MS. 222 1/2000 for 30 min. Fig. 10, stovaine 0·5 % for 30 mi Fig. 11, eucaine 0·5 % for 30 min. Time scale in Fig. 8, 1 per 2 sec.; time scales Figs. 9–11, 1 per 5 sec.

alized stimulation were then inserted into the nerve cord in the mid-ventral face of segment XII, and the preparation was subjected to prolonged eated stimulation. It was found that normal animals responded to



. 12. Luminescent response of a specimen narcotized with cocaine hydrochloride (0·5 %) for 52 min. The upper curve is the response under anaesthesia; the lower curve, the response after washing out the anaesthetic. Stimulation, 10 sec. burst at 5 per sec. Time scale above, 1 per sec.





s. 13, 14. Luminescent responses of specimens when anaesthetized, and after recovery from anaesthesia. The upper curve in each record is the response under anaesthesia; the lower curve, the response after washing out the anaesthetic. Fig. 13 (*above*), procaine hydrochloride 4 % for 28 min. Stimulation, 1 sec. burst at 5 per sec. Fig. 14 (*below*), isotonic $MgCl_2$ for 15 min. Stimulation, 22 sec. burst at 5 per sec. Time scale in both records, 1 per 5 sec.

mulation of the nerve cord by a bright flash along the aliform notopodia d by the release of luminous secretion in the light glands at the bases of ose structures. After anaesthetization, however, repeated stimulation failed produce any light.

This is strong evidence that the anaesthetics employed do actually stop nervous transmission, while allowing the peripheral effectors to respond directly to electrical stimulation. If the assumption be made that the anaesthetics have little or no effect on the activity of the glandular effectors, certain conclusions can be drawn about the effect of electrical stimulation of the light glands on the aliform notopodia as carried out in this investigation. Direct stimulation of the effector cells apparently produces much less light than stimulation of the nerves supplying them. This may be due to the effectors having a higher threshold than the nerve fibres so that, with a given voltage, fewer glandular cells would be affected than nerve fibres. Or it may result from the spatial organization of the nerve fibres themselves such that very localized stimulation of peripheral nerves initiates impulses that spread through a widely distributed network (not necessarily non-synaptic), and thereby reach the entire glandular area. In either event it follows that electrical stimulation of the peripheral light gland causes excitation of the nerve fibres supplying the glandular cells as well as direct excitation of the effector cells. But the major part of the light is due to indirect stimulation of the light cells via the nerve fibres, and only a small fraction is ascribed to direct stimulation of the effector cells themselves.

The Effect of Raising the Voltage in Anaesthetized Specimens

If the slight luminescence of anaesthetized specimens is due to a higher threshold in the responding structure or to the small field directly affected it is to be expected that a greater response could be secured by raising the voltage. With this in mind experiments were carried out as follows. Animals were anaesthetized in isotonic $MgCl_2$ and the light glands in the aliform notopodia were then stimulated at different voltages (charging voltages of the condenser, 9 V.–210 V.). A relay-operated stimulator activated by an electronic device was employed for these experiments. Luminescent responses at low voltages were small, but were greatly increased by raising the voltage (Fig. 15).

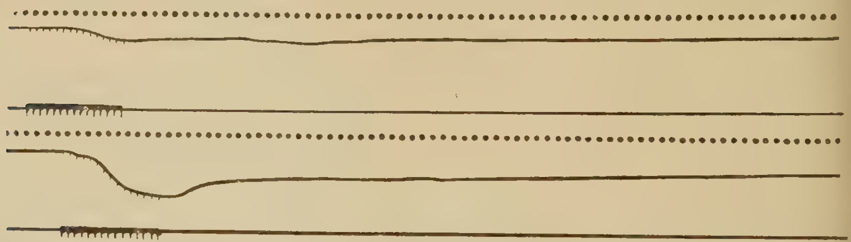
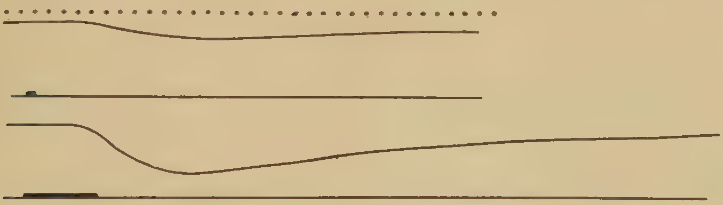


Fig. 15. Records of light produced by the photogenic glands of segment XII, demonstrating the effect of raising the stimulation-voltage in anaesthetized animals. The preparation was narcotized with isotonic $MgCl_2$ for 28 min., and it was then stimulated at 9 V. (*upper record*), and 49 V. (*lower record*). Stimulation consisted of a burst of 15 pulses a 84 per min. Time scale above each record, 1 per sec.

reover, by raising the voltage it was possible to secure responses equal in gnitude to those obtained from normal animals. These results favour the erpretation that in anaesthetized animals the gland cells are being directly nulated and that raising the voltage overcomes the higher threshold of se elements, or brings more into activity.

e Effect of Prolonging the Period of Stimulation in Anaesthetized Specimens

The action of narcotics in blocking nervous transmission while still owing the glandular cells to react presented the possibility of testing the ponses of the effectors to direct stimulation for varying periods. Animals re narcotized with isotonic $MgCl_2$ (pH 8·2) and MS. 222 (1/2000), and the ht glands of the aliform notopodia were then stimulated through electrodes ced directly upon them. Stimulation consisted of short bursts at 5 per sec., t for 1 sec., then again for 10 sec. Considerably increased amplification s used in recording the responses. In these preparations it was found that second period of more prolonged stimulation produced a much greater ninescent response (Fig. 16).



16. Response of the light glands of the aliform notopodia in a specimen narcotized with MS. 222 (1/2000) for 11 min. The light glands were stimulated directly. Upper curve, response to a 1 sec. burst at 5 per sec.; lower curve, response to a 10 sec. burst at 5 per sec. Time scale above, 1 per 2 sec.

It is emphasized that the luminescent responses recorded from these rcotized animals were very weak, and were only a small fraction of those tained from normal animals. The explanation is advanced that the small sponse is due to the excitation of only a small number of gland cells under ect stimulation. The fact that the same kind of augmented response can be tained both in narcotized and in untreated animals by increasing the number stimuli suggests that the processes involved are taking place peripherally, the effector cells, and not in efferent nervous pathways.

ght Produced by Stimulation of the Nerve Cord in Normal Animals

For comparison with the effects of peripheral stimulation some experi- ents have been carried out in which the nerve cord has been stimulated d the resultant luminescent response recorded. The arrangement was nilar to that described above, p. 119. The animal was spread out with dorsal side downwards: the basal light glands of the aliform notopodia overlay an aperture which was focused on a photocell lying below. Fine silver wire electrodes, giving localized stimulation, were inserted into the nerve cord on the median ventral surface, and stimuli were applied. Periods of stimulation were regulated so that the effects of short and prolonged bursts could be compared.

These experiments gave results similar to those obtained by stimulating peripherally, with the electrodes placed on the surface of the light glands. Two or three stimuli were delivered at a rate of 3 per sec., the response was recorded, and the light was allowed to fade; then a burst of thirty to forty stimuli at the same rate was applied, and a record was made of the response. In the majority of specimens (four out of five) the response to the second prolonged burst was appreciably greater than the previous response to fewer stimuli; in one specimen it was about the same (Fig. 17).

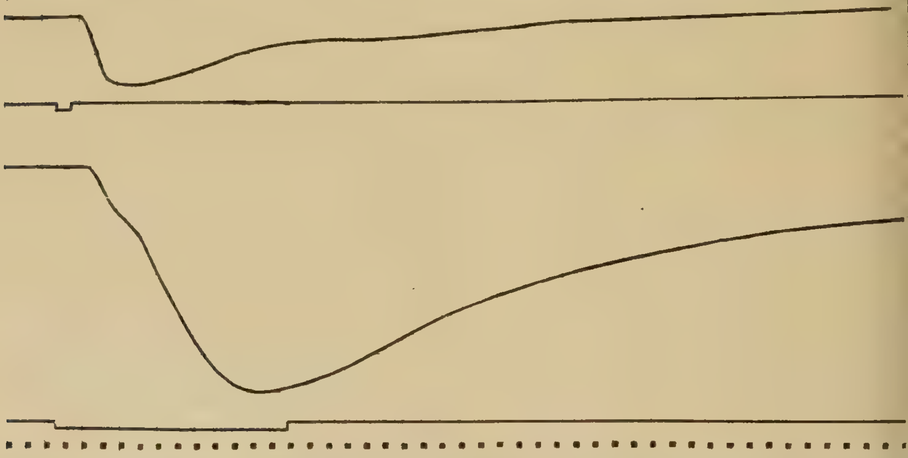


Fig. 17. Light produced by the aliform notopodia as the result of localized electrical stimulation of the nerve cord in segment XII. Upper tracing, three stimuli; lower tracing, thirty-eight stimuli. Frequency of stimulation, 3·3 per sec. Periods of stimulation are shown as horizontal intervals below each record. Time scale below, 1 per sec.

This is considered to be significant in view of the onset of fatigue or exhaustion previously discussed. The salient point that emerges from these results is that an augmentation of the luminescent response can be obtained by prolonging the period of nervous stimulation, that is, an increase in the number of nerve impulses brings about a greater response and produces more light. This result, read in conjunction with the previous conclusions relating to peripheral stimulation, lends weight to the viewpoint that when stimuli are delivered to the periphery they excite nerve fibres which in turn affect the glandular cells; reasons are presented above for considering that direct stimulation of the effector cells is a minor consequence. This point is of some importance in the present investigation since the animal does not lend itself readily to stimulation through the nerve cord, with simultaneous recording of the luminescent response, and the majority of quantitative results were obtained by applying stimuli through electrodes resting on the basal light glands of the aliform notopodia.

The Responses of Specimens from which the Nerve Cord had been Removed

The reverse experiment to the preceding one has been carried out, namely stimulation of the peripheral light glands without involving the central nervous system (nerve cord). In the usual experimental arrangement, with electrodes resting on the dorsal surface of the animal, it is likely that solely efferent paths are involved rather than the nerve cord; but excitation of the latter, and consequent central regulation of the response, are not excluded. The following experiment was undertaken to resolve this difficulty, and the results indicate that the events responsible for augmentation of the light response on increasing the duration or frequency of stimulation occur at the periphery.

The worms used in these experiments were first anaesthetized by immersion in isotonic $MgCl_2$ for 15 min. The anterior regions were cut off at the junction between segments XII and XIII, and the ventral surface was removed from these anterior fragments. This operation removed the nerve cord (central nervous system) and left pieces which consisted of dorsal body wall and gut, and which contained the light glands of segment XII. The experimental material was afterwards washed in running sea water for several hours. These pieces were then stimulated either at two different frequencies or with short and long bursts of stimuli.

This experimental material showed the same responses as intact worms containing central nervous system. The response to a second period of stimulation at a higher frequency was significantly greater than after the first burst of stimuli at a lower frequency (Fig. 6). The response was also augmented by increasing the number of stimuli (Fig. 18). Both the maximal height of response and the total light produced were greatly increased by stimulating at a higher frequency or for a longer period. In addition, the rate of rise of light intensity showed a pronounced increment. It follows that the augmentatory effect, whatever its nature, that is produced by more stimuli or faster rates of stimulation, can be a peripheral affair, and experimentally is not dependent on the central nervous system and centrally located nerve cells.

Histological sections of the aliform notopodia, impregnated with silver (Holmes's method and Bodian's activated protargol), have revealed some details of innervation. The nerve cord, situated mid-ventrally, gives off small nerves which proceed peripherally underneath the epidermis. The nerve fibres in these nerves are very small and it has not been possible to distinguish terminal details of peripheral innervation, but the multiplicity of fibres and their close proximity to the glandular photogenic cells form a structural basi for the physiological mechanisms reported above.

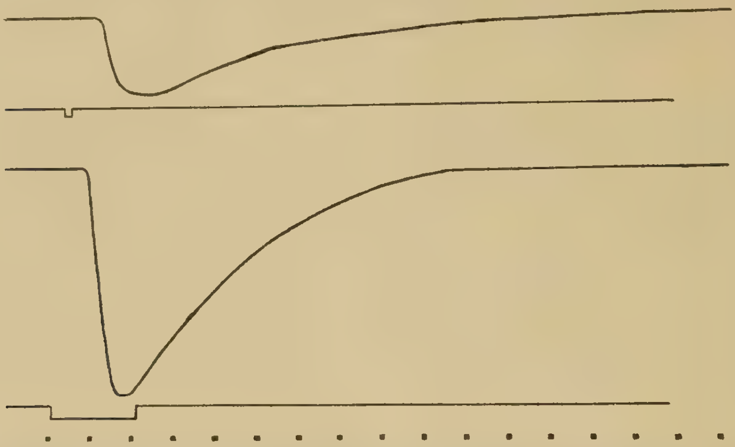


Fig. 18. Record of light produced by the light glands in the aliform notopodia of a preparatio from which the nerve cord has been removed. Upper record, three stimuli; lower record 10 sec. burst (fifty stimuli). Frequency of stimulation, 5 per sec. The tracing below eac record indicates the period of stimulation. Time scale below, 1 per 5 sec.

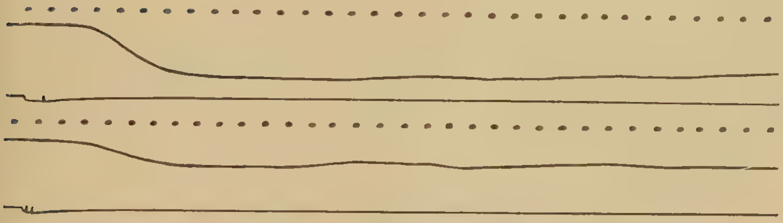
Comparison of the Effects of One versus Two Stimuli

In order to secure critical data on whether the augmentation of the ligh response is due to a process of summation in the effectors (luminescent glan cells), or to facilitation at the neuro-effector junction, specimens have bee stimulated with a single stimulus and with a pair of stimuli, and the response compared. Two sets of experiments were carried out as follows.

In the first set of experiments each animal was stimulated twice. It wa stimulated by a single impulse; then, after allowing a period for decay, tw electrical stimuli were applied. The two impulses were separated by a interval of 0·25 sec. In these experiments all the results obtained wer negative. Of seventeen specimens examined a few gave about the sam amount of light with two stimuli as with one; the majority, however, gave of even less light following the second period of stimulation (Fig. 19). It wa concluded that a fatigue effect was operating here, in that the first stimulu had partially exhausted the light glands; any augmentative effect due to tw stimuli would be masked by the previous partial evacuation of the light glands

A second approach to the problem was essayed by stimulating separat specimens either with a single stimulus, or with two stimuli (interval 0·25 sec.) Forty-six animals were used, half of which were stimulated with a singl impulse, and half with two stimuli. Since the experiments lasted severa hours and there was the possibility of an alteration in the experimenta conditions during that time, the animals were divided into small groups. I ch group several animals were stimulated with one stimulus, and a correonding number with two stimuli. The mean response of each group was termined, and from these values weighted means were calculated for the hole assemblage. (Measurements of response refer to displacement of the sponse curves.)

Group	Mean responses of each group (in arbitrary units)	
	one stimulus	two stimuli
1	10·0	5·2
2	2·9	4·3
3	11·4	13·7
4	10·3	12·0
5	9·6	5·4
6	10·2	7·7
Weighted means	8·3	7·8



z. 19. Records of light produced by the photogenic glands on the aliform notopodia. *Upper record*, the response to a single stimulus; *lower record*, the response of the same specimen to two stimuli (interval of 0·25 sec.). Stimulation indicated below each record. Time scale above, 1 per sec.

To reduce the possibility that some partly exhausted specimens were iating the results, a comparison was made of the twenty maximal responses. eans for these were 14·2 units for one stimulus (ten animals) and 12·5 units r two stimuli (ten animals).

In these experiments two stimuli produced rather less light than a single pulse. The actual fact that the mean response was slightly less for two muli than for one, however, is certainly a statistical accident. If two muli were to give a disproportionate response, equal to many times that oduced by a single impulse, then it would be expected that it would be vealed by the technique employed, and might be sufficient even to offset rtial exhaustion resulting from a previous stimulus. Other records demonate, moreover, that a series of repeated stimuli do give a significantly eater response than one or a few stimuli (Figs. 2, 7, 15, 16).

Facilitation due to build-up of an excitatory state seems to be ruled out by se results, but they can be explained by summation in a contractile tissue the light glands. They suggest a mechanism in which the first stimulus ngs about a large response (or contraction) and subsequent stimuli are reonsible for only small additional increments. This explanation of peripheral mmation is in line with the results of experiments reported above (p. 123), in which augmentation of the light response was obtained by stimulati of the photogenic glands in narcotized animals, presumably due to dire excitation of the effector-gland cells. The subject is discussed more fully a later section.

Rate of Rise of Light Intensity and Temporal Characteristics of the Luminescent Response

The augmented responses observed in some of the experiments describ above obviously invite comparison with results obtained in investigations neuromuscular functioning. From experiments on crab muscle Pantin (193 was able to show that as the frequency of electrical stimulation was raise there was a corresponding increase both in the maximal tension develop and in the rate of rise of tension. These studies in neuromuscular functioni have prompted further analyses of the light response of *Chaetopterus*.

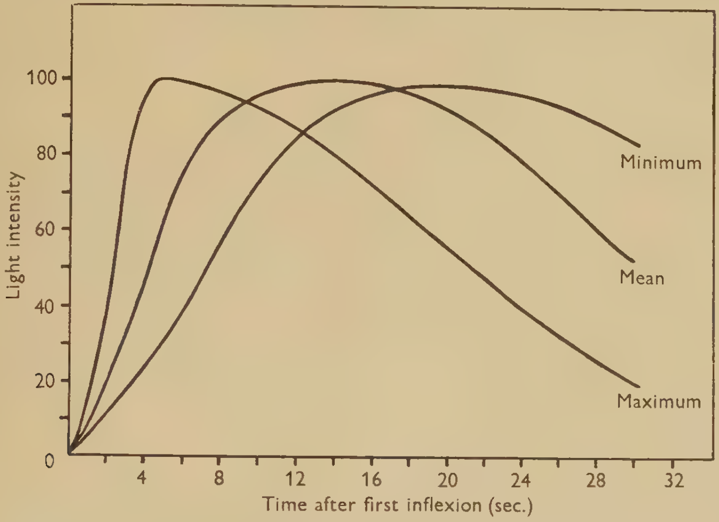
Measurements made on those records that show an increased response a higher frequency of stimulation, or to more prolonged stimulation, al reveal a corresponding increase in the rate at which the luminescent respon develops (Figs. 4, 7). Data from five experiments in which the frequency stimulation was raised are presented below.

Specimen	Frequency of stimulation (per sec.)	Rate of rise per sec. (mm.)
1	1·3	4·0
	9·0	8·9
2	1·3	1·7
	9·0	2·8
3	1·3	1·3
	9·0	3·8
4	2·0	3·0
	9·0	4·9
5	5·5	2·4
	32·2	4·9

Mean increment at lower rate, 2·5 mm./sec.
Mean increment at higher rate, 5·1 mm./sec.

In these experiments the frequency of stimulation was raised 1/2–7 tim and the rate of rise of light intensity increased about twofold at the high frequencies. The rate of rise of light intensity is also significantly increas by increasing the number of stimuli (Fig. 7). An increased response, ther fore, reveals itself in three ways: by a rise in peak intensity, by an increase the rate of rise of light intensity, and by an increase in the total amount light.

Detailed analyses of a large number of response curves have yield information about certain temporal features of the light response and the ra of increment of light intensity. The following data were extracted from ea record: the rate of rise of the response curve, the time taken to reach maxim height, the maximal height of the response, and the rate of decay for a peri xtending up to 30 sec. after the beginning of the response. Due to the great riation between individual specimens, it has been necessary to reduce the cords to a comparable basis, and this was accomplished by expressing all tensities as a percentage of the maximal response, set at 100. Fig. 20 shows representative plot for a series of nineteen specimens. In this figure two rves represent records which showed maximal and minimal rates of crement, and a third curve represents mean values for all specimens.



g. 20. Curves showing temporal characteristics of the luminescent response evoked by electrical stimulation (2·3 sec. burst at a frequency of 6·2 per sec.). Maximal and minimal curves are selected records showing fastest and slowest rates of rise of light intensity. The mean curve is based on the average for all records (19). All data have been replotted as a percentage of maximal response (=100).

ean values obtained in this series of experiments were:

Time to reach maximal height, 14·2 sec.;
Rate of rise, 13·1/100 per sec.;
Height at first ½ min., 53·8/100.

hese figures illustrate the levels of magnitude involved, but absolute values ill depend on the conditions of stimulation.

The relationship between the rate of rise of light intensity and the maximal tensity of response was considered in the following manner. Records were tained of sixty-eight animals which were stimulated at two different equencies, first at 2 per sec., and again at 25 per sec. The absolute increent (h_1) 5 sec. after the beginning of the response was measured, and this as expressed as a fraction of the maximal response (h_2), viz.

$$\frac{h_1}{5}\times\frac{1}{h_2}=r, \quad \text{the fractional increment per sec.}$$

A mean value for r was obtained by pooling measurements from all record The absolute rate of increment is sometimes greater at higher frequencie yet, when expressed as fractional increments of the total response, th rates of rise at low and high frequencies show no significant differenc i.e. $r_2=r_m=r_{25}$, where r_2=fractional increment at a frequency of 2 per sec r_{25}= fractional increment at a frequency of 25 per sec., and r_m=the mea fractional increment of all records irrespective of the frequency of stimulatio This result indicates that the rate of rise of light intensity (i.e. the rate cellular secretion), and the maximal intensity reached (i.e. the maximal ou put of secretion) are related to one another.

Effect of Drugs on the Neuro-glandular Junction

The results just described reveal the characteristics of certain excitato events taking place in the light glands. It has also been shown that acety choline is an effective agent in causing luminescence in this animal, and ac at the neuro-glandular boundary (Nicol, 1952*b*). In conventional terminolog the nerve fibres to the light glands are therefore cholinergic, and acetylcholi becomes implicated as a chemical transmitter in the light response. In vie of these facts it seemed of value to test the effects of atropine and curar which separately antagonize the action of acetylcholine in different neur effector systems of vertebrates.

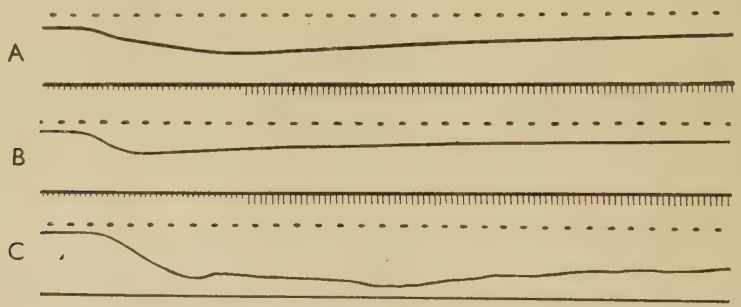
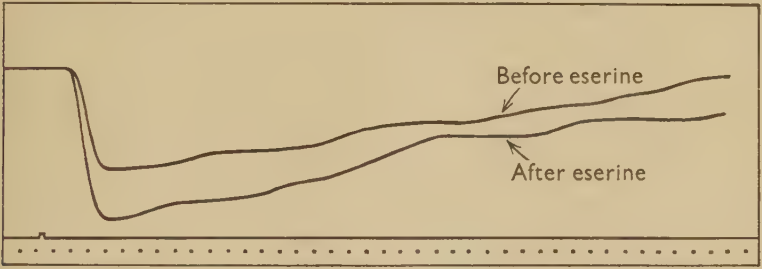


Fig. 21. Records of the light produced by animals treated with atropine or curare and subject to electrical stimulation (10 sec. burst at 4 per sec.). A, specimen treated with atropin 1/10,000, for ½ hr., and then stimulated; B, same specimen stimulated after washing o atropine for ½ hr.; C, specimen treated with *d*-tubocurarine, 1/10,000, for ½ hr., and th stimulated. Neither atropine nor *d*-tubocurarine had any effect in abolishing the lig response. Time scale, 1 per sec.

Atropine. This drug was applied in concentrations of 1/10,000 ar 1/1000 for 30 min., and records of the luminescent response to electric stimulation were obtained. The drug was then washed out for 30 min., ar stimulation was repeated. The drug had no apparent effect in reducing th amplitude of the response (Fig. 21).

d-*tubocurarine.* At a concentration of 1/10,000, *d*-tubocurarine failed iminish or abolish the luminescent response evoked by electrical stimulation Fig. 21).

Eserine. Some previous experiments with eserine failed to reveal any xcitatory effect of this drug when used in conjunction with acetylcholine. Iowever, little reliance is placed on this negative result, since the light roduced by acetylcholine itself is usually very faint and shows much variation rom one specimen to another. The effect of eserine has been further explored a quantitative manner by stimulating specimens electrically to determine ne normal level of response, and then stimulating again after the application f eserine to discover any possible augmentative effect. Such an experiment uffers from the same defects as those mentioned previously in describing ne effects of increasing the frequency or duration of stimulation, namely atigue of the light glands. Despite this latter factor an increase in response fter eserinization might still be encountered, and such a result may be conidered as positive.



g. 22. The effect of eserine on light production in *Chaetopterus*. The upper tracing shows the response to electrical stimulation before eserine, the lower tracing after the application of eserine (1/10,000, 45 min.). Electrical stimulation, in both instances, was a 0·2 sec. burst at 33 per sec. Lower tracing: stimulation, and time scale, 1 per sec.

Specimens of *Chaetopterus* were treated with eserine (physostigmine alicylate) 1/10,000 for 45–60 min., and the light produced before and after serinization was recorded and compared. Of twelve animals examined, three ave positive results in that a brighter response followed the application of serine (Fig. 22).

Acetylcholine. It was considered possible that the luminescent responses to ectrical stimulation might be affected by acetylcholine, and experiments ong this line were carried out as follows. A record was first obtained of the ght resulting from a short burst of electrical stimuli. The animals were then mmersed in 1/10,000 acetylcholine for 15 min., and stimulation, as before, as repeated. In all instances (five specimens) the light resulting from the cond period of stimulation was less than that obtained previously. There as, consequently, no evidence that acetylcholine applied externally can crease the luminescent response that is evoked by stimulation of the nerves.

The negative results with atropine and *d*-tubocurarine do not directly provide any information about the nature of neuro-glandular transmissio occurring in the light glands. However, other cases are known amon invertebrates in which cholinergic systems are insensitive to these depressiv drugs. Presumably, the receptor surfaces involved in neuro-muscular transmission are not affected by these substances, and the reactant systems consequently do not readily lend themselves to classification as muscarinic o nicotinic. The results obtained with eserine are interesting and point toward acetylcholine being involved as a chemical mediator in the light glands.

Transmission Through the Nerve Cord

In a previous study it has been shown that when the frequency of electrica stimulation is raised above 5 per sec., the luminous response spreads an affects more distant segments. The transmission of excitation involved in th process occurs through the nerve cord (Nicol, 1952*b*). The passage of excitation from one body region to another is rather irregular in occurrence. Fo example, it is only occasionally that light appears in the middle and posterio regions of the body when the anterior region is stimulated. In order to obtai repeatable quantitative information, therefore, experiments were confined t the posterior region.

The posterior regions of a series of specimens were stimulated at variou frequencies in the dark, and the number of posterior segments that lighte was noted. The electrodes were placed on the first few segments (I–III) of th posterior region, and the voltage output of the stimulator was kept constan The effect of increasing either the number or frequency of stimuli wa investigated. A diagrammatic representation of the results is shown in Fig. 2

Increase in frequency of stimulation. There is no spread of excitation at low frequencies. Stimulation at 1–2 per sec. results in light confined to the notopod in the neighbourhood of the electrodes. On raising the frequency to high rates, excitation spreads to involve more distantly located segments, and th whole posterior region finally lightens. The critical frequency is abo 6 per sec.

Increase in duration of stimulation. Prolonged stimulation at low frequencies (1–2 per sec.) does not widen the response area, but at high frequencies (above 5 per sec.) the spatial character of the response depend upon the duration of stimulation as well as the frequency (Fig. 23). Tw protocols illustrate the results obtained.

Specimen 1. Electrodes on segments I–II of the posterior region.
Stimulation: 2 per sec.
Time: 10 sec. Segments I–II responded.
22 sec. Segments I–IV responded.
22 sec. Segments I–IV responded.
Stimulation: 25 per sec.
Time: 20 sec. Bright luminescence from all segments of the posterior region.

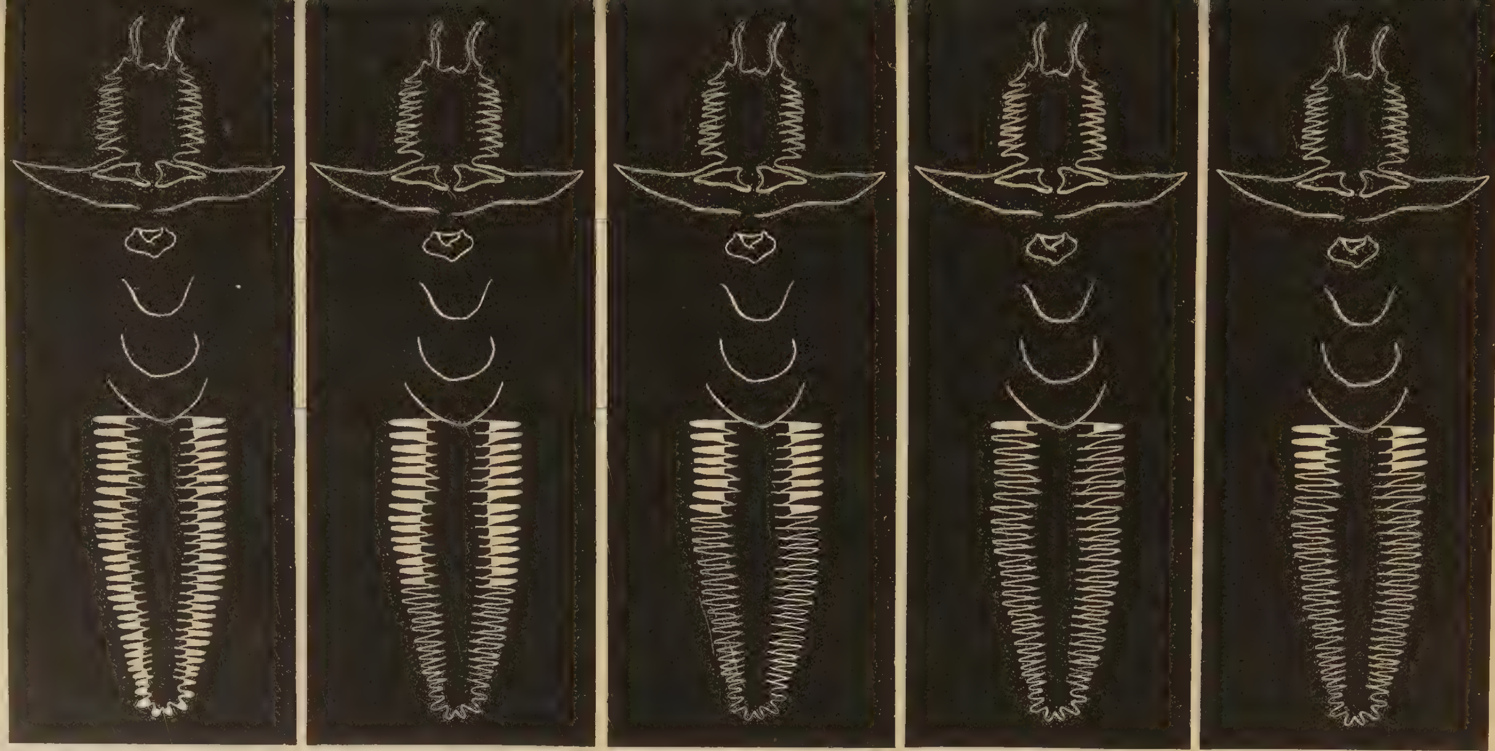


Fig. 23. The effect of altering the frequency and number of impulses on lighting of the posterior region of *Chaetopterus*. A, electrodes on segments I and II. Stimulation, 43 per sec. for 10 sec. Luminescence in all notopodia of the posterior region. B, electrodes on segments II and III. Stimulation, 10 per sec. for 10 sec. Luminescence in segments I–XV. C, electrodes on segments II and III. Stimulation, 10 per sec. for 2 sec. Luminescence in segments I–VIII. D, electrodes on segments I and II. Stimulation, 10 per sec. for 0·2 sec. Luminescence in segment I. E, electrodes on segments I and II of the posterior region. Stimulation, 1·2 per sec. for 40 sec. Luminescence in segments I–IV.

Specimen. 2. Electrodes on segments I–II of the posterior region.

Stimulation: 43 per sec.
Time: 0·2 sec. Segments I–II responded.
0·5 sec. Segments I–II responded.
1 sec. All posterior segments responded, but light weak.
10 sec. All notopodia of the posterior region were briefly luminous.

This spread of excitation is interrupted by cutting the nerve cord, and is therefore transmitted through the central nervous system. To explain these results it has been suggested that some process of internuncial facilitation occurs at higher frequencies (Nicol, 1952*b*). The present results show that the spread of excitation is also dependent upon the number of stimuli, and as the duration of the stimulatory period is increased, the luminous response spreads so as to involve more and more posterior segments.

Slightly different results are obtained by stimulating segments in the middle or hind end of the posterior region. When the middle of the posterior region is stimulated at low frequencies (1–5 per sec.) the appearance of light is usually confined to notopodia lying underneath and posterior to the electrodes. But on raising the frequency and number of impulses the light spreads to involve notopodia lying anterior to the electrodes. Here are two protocols.

Specimen 3. Electrodes on the terminal third of the posterior region.

Stimulation: 1 sec. burst at 5 per sec. Segments from the level of the electrodes to the hind end responded.
22 sec. burst at 5 per sec. All segments of the posterior region lighted up, anterior and posterior to the electrodes.

Specimen 4. Electrodes on the middle of the posterior region.

Stimulation: 3 per sec. for 20 sec. Only notopodia behind the electrodes responded.
5 per sec. for 20 sec. Only notopodia behind the electrodes responded.
10 per sec. for 20 sec. All segments of the posterior region responded.

In most of these animals it was observed that the light appearing anterior to the region stimulated was much weaker than that appearing posterior to that region.

The conclusion drawn from these experiments is that nervous transmission concerned in mediating the luminescent response takes place with much greater facility posteriorly than anteriorly through the nerve cord. Although transmission anteriorly does occur, there is a greater degree of resistance to it in postero-anterior pathways than in the reverse direction.

The build-up of a central excitatory state is presumably a synaptic phenomenon occurring at the junctions between segmentally disposed neurones arranged in linear sequence along the nerve cord. It may be assumed that at low frequencies, below 5 per sec., the excitatory state dissipates in the intervals between stimuli, but that at higher frequencies the rate of build-up of excitation exceeds the rate of decay. The degree of facilitation attained will depend on the frequency and number of impulses, both ctors determining the level of excitation reached. It appears that a single npulse, or a group of impulses at low frequencies, is insufficient to override ie synaptic resistance between the segmentally arranged neurones, and that burst of impulses is necessary to overcome these barriers. The number of gments responding can be interpreted as the external manifestation of cilitatory processes occurring at sequential synapses, each being a barrier series to the next, and introducing a degree of decrement in stepwise series.

Correlated with this physiological interpretation it has been observed that ie nerve cord possesses large tracts of longitudinally directed fibres, coursing om one segment to the next. Besides minute fibres, giant axons have also een noted, up to 7 μ in diameter. These probably only extend short distances, owever, since Bullock (1948) could find no electrical evidence for giant fibres this species.

Discussion

eripheral augmentation of the luminescent response due to increasing the equency or duration of stimulation manifests itself in heightened light tensity, often in augmentation of the total amount of light produced, and an acceleration in the rate of increase of light intensity. The light produced taken to be an adequate indication of the strength and temporal progress of ents in the underlying response mechanisms. The response curve, of course, presents the progress of some chemical reaction, presumably the oxidation photogenic material, and it only indirectly reveals the course of the nysiological processes of secretion. To interpret this curve it is necessary consider its relationship to the secretory process. Unfortunately, there are critical biophysical data available for the oxidation of the luminescent ecretion of *Chaetopterus*, and experiments with *Cypridina* (Ostracoda) tracts can only be cited for comparison. Harvey and his colleagues (1940) udied the luminescent reaction produced by mixing *Cypridina* extracts, d they found that the light emitted by this material reached its maximum in bout 0·03 sec., and the decay time for a fall to half intensity occupied 0·5 sec. hese experiments were carried out with refined extracts, and it is obvious at the reaction in *Cypridina* preparations proceeds at a much faster rate an in the luminescent material of *Chaetopterus*, as released by the normal imal (see p. 129). The secretion of *Chaetopterus* does not become luminescent ntil it is discharged from the cell and this in itself must introduce a time lag that the luminescent response is not contemporaneous with the secretory ocess. Under optimal conditions it may be presumed that the chemical action will proceed rapidly to completion, but the luminescent secretion of ie light glands in the aliform notopodia is actually released into a dense body mucus that greatly retards gaseous diffusion, and, consequently, the oxidaon of the photogenic material.

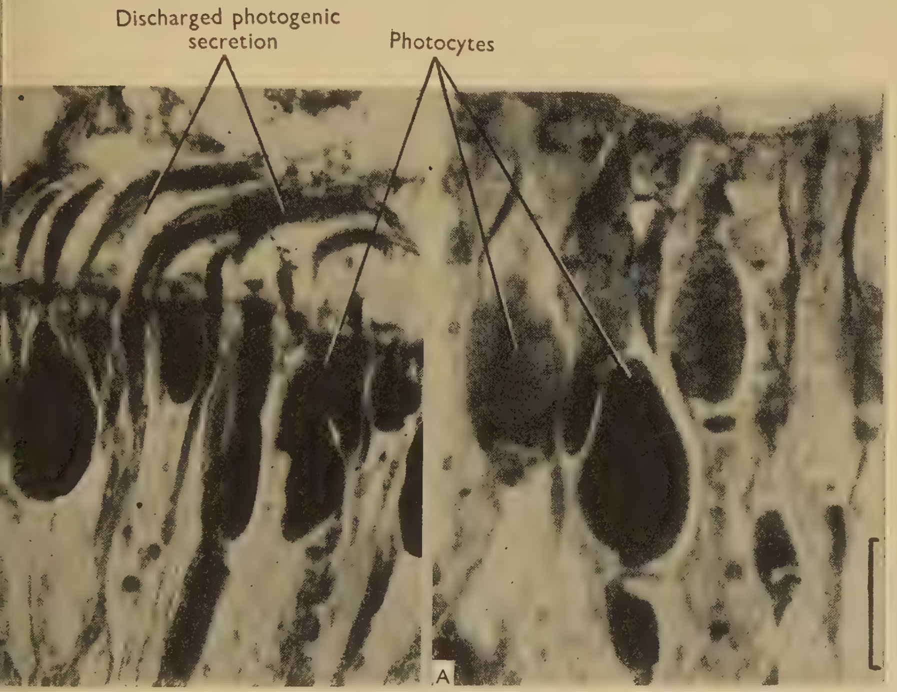
A study of the latent period is more informative. When the aliform notopodial light glands are stimulated, the average latent period is 4 sec., as determined by measurements of the response curves. This latent interval of 4 sec. could be occasioned by the latent period of the effector, by the time consumed in the response of the effector, and by delay in the course of the chemi-luminescent reaction. In several experiments in which the effect of raising the frequency was investigated, stimulation consisted of short bursts of 0·5–4 sec. (p. 116). With these short bursts, lying within the latent period a definite augmentation of the response was obtained on increasing the rate of stimulation. Other evidence indicates that augmentation of the response is due to summation. Since this process can take place well within the latent period of the response, it is clear that the luminescent response first becomes evident several seconds after the sequence of events in the underlying mechanism has taken place. On these premises it is now possible to predicate that most of the interval that elapses between the delivery of the first electrical shock, and the first appearance of light, is due to the slow progress of the chemiluminescent reaction in the material discharged upon the surface of the animal.

Since luminescence becomes evident considerably later than the sequence of events taking place in the underlying mechanism of secretory discharge the response curve will not reveal detailed information about the temporal course of events in the secretory structures. On the other hand, its heigh and slope reflect the magnitude of the underlying secretory processes, and give a delayed, smoothed, and aggregate portrayal of the events preceding it

The cytomorphological appearance of the photogenic glands is that of ova or elongated cells closely packed with eosinophilic granules (see Nicol, 1952*a* for details and references to literature). These are arranged as a closely packe mass of paraplasm occupying most of the cell interior, and invested by a thi layer of cytoplasm. The mechanics of secretion in such a cell appear to b poorly understood, and present a problem common to many glandular cell of that type, for example, muciparous cells containing a mucous plug. I may be suggested that excitation causes the superficial protoplasm of the cel to contract, and this process leads to the expulsion of the cell contents; or tha excitation initiates changes resulting in imbibition of water and a rise in interna pressure, again causing the photogenic mass to be squeezed forth. In eithe case nervous excitation of the glandular cell would be direct, and would resul in a rise in internal pressure. Other possible mechanisms that can be suggeste are the existence of myo-epithelial elements about the cell-body, as obtains i the oral glands of mammals, or of muscle elements between the epiderma gland cells as Eisig (1887) has described in the epidermis of capitellids The mechanism in these instances would be indirect and neuro-muscular i nature.

In conjunction with the physiological investigations a considerable amoun of histological work has been done on the photogenic glands and cells c *haetopterus*. Specimens, fixed in a variety of ways, have been stained and npregnated with silver by silver-on-the-slide techniques. This work is mmarized as follows.

The thin layer of protoplasm investing the photogenic cells stains rather ebly, probably owing to its tenuity. In preparations triple-stained with eidenhain-azan this protoplasmic investment is coloured orange or reddish ke the muscle fibres in the same sections. Treatment with silver (Holmes's ethod after Bouin fixation) sharply delimits this layer as a distinct deeply



g. 24. A, section across an aliform notopodium to show some photogenic cells. Bouin fixation; impregnated with silver by Holmes's method. B, section across a notopodium of the posterior region to show photogenic cells caught in the act of secreting. Fixative, formol-sea water; stain, Heidenhain-azan.
The scale in both sections corresponds to 25 μ.

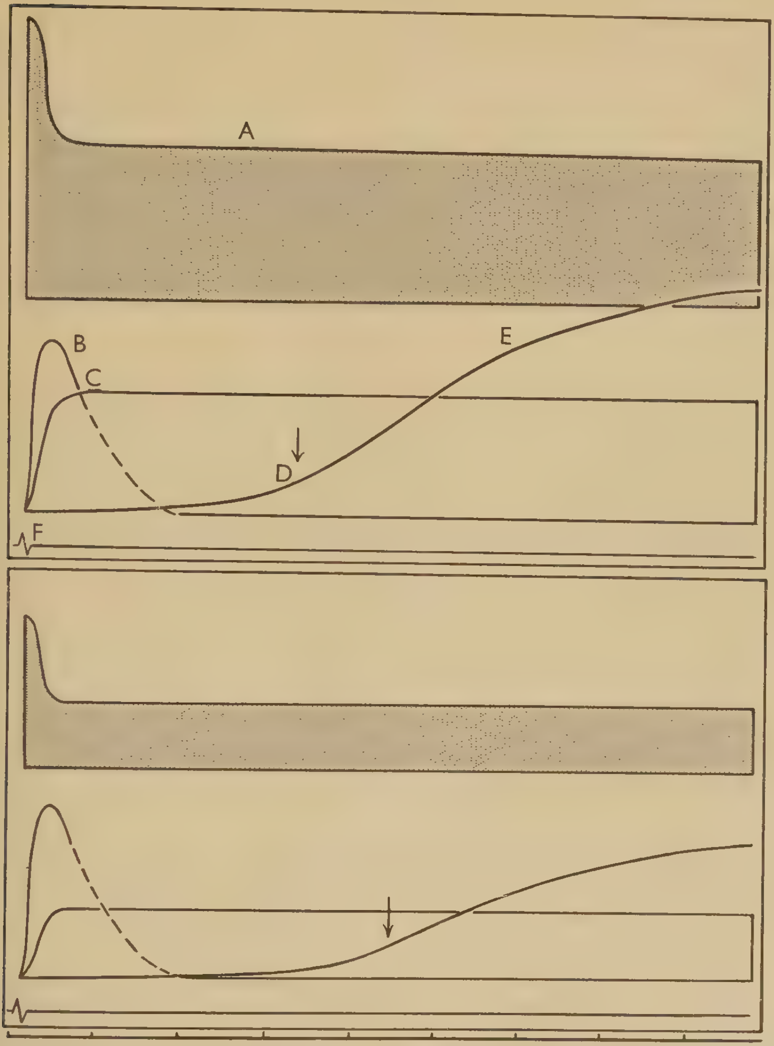
npregnated sheath about the photogenic material (Fig. 24A). The possibility at the protoplasmic investing layer contains fibrous oriented protein similar that in muscle was considered, and sections were examined in polarized ght. This material had been fixed in formalin or Bouin's fluid and was amined unstained. Under crossed Nicols and with a red gypsum plate the hotogenic cells displayed no anisotropy although muscle fibres in the same ctions were markedly birefringent. It was noted, however, that the protoasmic layer about the photogenic cells in these unstained preparations was strongly refractile. Moreover, careful examination of the epidermis has provided no evidence for the existence of myo-epithelial cells or epidermal muscle fibres.

This study, unfortunately, is inconclusive, but some preparations were obtained in which the photogenic cells were caught in the act of discharging their secretion (Fig. 24B). These sections give the impression that the photogenic material is being forcibly squeezed out of the cell. It is tentatively proposed that the protoplasmic investment of the photogenic cells is a contractile structure, capable of compressing and causing the evacuation of the photogenic cells when excited. The postulation of an organization of this kind permits the physiological results obtained in the present investigation to be interpreted as manifestations of the activity of contractile units, excited by nerve impulses, and such an interpretation permits comparison with results obtained in neuro-muscular physiology.

The augmented luminescent response obtained by increasing the number of stimuli or frequency of stimulation can be explained by a process of summation in the effector organ (light gland). Certain of the experiments were designed to reveal the possible existence of a facilitatory mechanism, with negative results. If facilitation were operating it would be expected that two stimuli would have an appreciably greater effect than one stimulus, even after allowing for partial exhaustion of the luminescent secretion. However, such an effect was not discovered.

The events that are considered to be taking place during stimulation of the photogenic glands on the aliform notopodia of *Chaetopterus* are depicted diagrammatically in Figs. 25 and 26, and are interpreted as follows. An electrical stimulus gives rise to a nerve impulse that activates the photogenic cells, causing them to contract, and results in the expulsion of enough secretion to produce visible luminescence. A single impulse causes a strong response that evacuates a large part of the cell contents. After this response has exhausted itself, a second impulse acting on the cell would initiate changes in the same response-mechanism, but since the paraplasmic contents of the cell are now only a fraction of their former level, the response will also appear as a fraction of the former intensity (Fig. 25). Subsequent stimuli, spaced at intervals so as to allow recovery of the effectors, should evoke responses of geometrically decreasing magnitude. This seems to be realized in actuality, and would explain the progress of fatigue under repetitive stimulation.

A series of impulses gives rise to a series of contractions which summate with one another to produce a heightened response (Fig. 26). A large part of the secretion would be expelled with the first contraction, and each subsequent contraction would have a relatively smaller effect. Two contractions then would produce only a little more secretion than a single contraction, and a whole series of contractions would be necessary to increase greatly the amount of luminescent material. Similarly, raising the frequency would



. 25. Diagrams intending to illustrate the possible series of events taking place during the luminescent response of *Chaetopterus*. It is postulated that a stimulus (shown on the bottom line) excites the nerve fibres supplying the light gland and causes the gland cells to contract (illustrated as the first response curve on the left). This brings about the discharge of photogenic material and causes partial evacuation of the glandular cells. Both the secretion of photogenic material, and the residual amount remaining in the cells, are represented. Some time after the secretion of photogenic material, the photochemical reaction attains sufficient velocity to be revealed as a measurable luminescent response (threshold indicated by vertical arrow). A second impulse, after the effect of the first has died away, results in less secretion since the secretory content of the glandular cells is less. A, content of photogenic material in gland cells. B, contraction of gland cells. C, amount of photogenic material secreted. D, threshold sensitivity of recording apparatus. E, luminescent response. F, stimulus. Time scale, 1 per sec.

result in greater secretion since the individual contractions could summate before relaxation ensued or became advanced.

The rate of rise of the luminescent response curve remains to be dealt with. The slope of the curve becomes steeper as the frequency and number of impulses are raised, and it is related to the height of the response, i.e. an increase in the rate of rise of light intensity is followed by an increase in the maximal light intensity reached. This is probably due to the fact that as the contractile response becomes stronger, more secretion is poured out, and the velocity of the ensuing chemiluminescent reaction is governed by the initial concentration of the photogenic material. If this is so, it would be expected that the latent period would be reduced when the response becomes greater, and this is borne out by inspection of records of augmented responses. In Fig. 7A, for example, the latent period was reduced from 4·6 to 3·4 sec. when the duration of stimulation was prolonged from 0·2 to 5 sec.

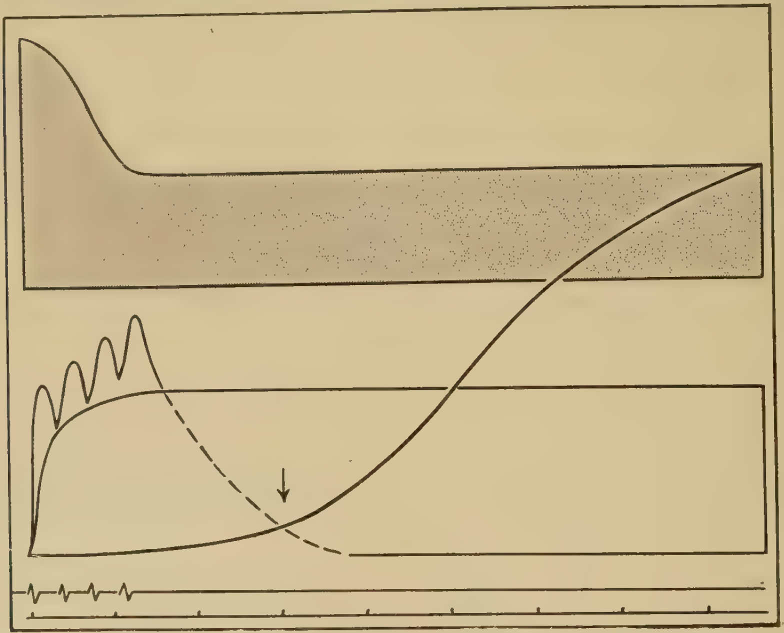


Fig. 26. Diagram, as in Fig. 25. A burst of impulses produces summation and augmented secretion.

The salient fact that emerges from this investigation is that the luminescent reaction is a triggered response. In the light glands of segment XII, a single impulse as well as a burst of impulses evokes luminescence. It may therefore be concluded that any effective natural stimulus, sufficient to excite sensori-neural pathways, will produce a response, without the necessity of reinforcement by repeated stimulation. In some specimens of *Chaetopterus* it has been possible to produce an enhanced effect with a second period of stimulation t a higher frequency or duration, but in many of the animals investigated he second response was actually lower than the first, and fatiguing of the ght response is an invariable accompaniment of repetitive stimulation. Moreover, recovery from fatigue is extremely slow, a matter of hours rather han minutes. The nature of the effector organ itself, therefore, precludes the ossibility of the nervous system exercising any effective control over the evels of response on frequently repeatable occasions.

Although the magnitude of the initial response would depend on the conitions of stimulation, nevertheless, any excitatory stimulus results in the ppearance of light. It has been suggested that luminescence in the aliform otopodia of *Chaetopterus* may be in the nature of a sacrifice lure associated with autotomy of the anterior region (cf. Joyeux-Laffuie, 1890), and, under his interpretation, the significance of luminescence as a triggered response ecomes intelligible. It would be of value to the animal to produce a maximal mount of light initially, when stimulation reached or exceeded threshold, nd actual variations in a few millilamberts at the low levels of brightness nvolved, would probably have little survival value as long as the response xceeded the visual threshold of the photoreceptor concerned.

In contrast to the events occurring peripherally, where a single impulse roduces a pronounced luminescent response, the operation of facilitatory rocesses can be observed as the result of stimulating the nerve cord. These rocesses are manifested by an extension of the luminous area as the rate and uration of stimulation are raised. Since the response is observed as an xtension of the field of luminescence involving more and more glandular units, is not obscured by the intervention of glandular fatigue. The underlying mechanism seems to consist of a progressive increment of an excitatory state synapses in the nerve cord. Such a mechanism could obviously provide way of regulating the magnitude and extent of luminescence in posterior egments in response to natural stimulation, but the part that it might play in he normal life of the animal still awaits determination.

SUMMARY AND CONCLUSIONS

The nervous regulation of luminescence in *Chaetopterus* has been investigated y making use of controlled electrical stimulation and photoelectric recording. o achieve the latter a multiplier photocell has been used in conjunction with galvanometer or oscilloscope, and camera.

A luminescent response is evoked in the aliform notopodia by a single timulus or by a battery of stimuli. Due to the intervention of fatigue under epetitive stimulation it is difficult to obtain reproducible results, but in vourable preparations it has been possible to secure an enhanced response o increase in frequency and duration of stimulation above a rate of 1 per sec. imilar results were obtained by localized stimulation of the nerve cord, and by peripheral stimulation of preparations from which the nerve cord had been removed. It is, therefore, concluded that the photogenic glands are being stimulated through their nerve fibres, and that the enhancement or augmentation of the response is not dependent upon the central nervous system, but takes place peripherally in the glandular tissue itself.

In experiments with two closely spaced stimuli no greater response was obtained than with a single stimulus. After making allowance for individual variation in the intensity of the response and partial exhaustion of the light glands following one initial stimulus, these results are interpreted as demonstrating that peripheral facilitation plays no part in the luminescent response. Any augmentation of the response due to prolonged or high frequency stimulation can be explained satisfactorily by summation in the effector organs.

The blocking effect of a number of anaesthetics on the luminescent response was determined. Chloretone, MS. 222, stovaine, procaine, eucaine, cocaine and isotonic magnesium chloride all greatly diminish the luminescence which can be evoked by peripheral stimulation, and prevent excitation of the photogenic glands when the nerve cord is stimulated. Ether at 0·1 % had little effect. The small response under anaesthesia to peripheral stimulation could be progressively increased by raising the voltage. It is concluded from these results that the anaesthetics block nervous transmission while still allowing the glandular cells to respond directly to electrical stimulation. The assumption is made that the anaesthetics do not materially reduce the responsiveness of the glandular cells. It follows that peripheral stimulation achieves its effect in large part through nervous excitation, but a small amount of direct excitation of the gland cells is also involved.

Augmentation of the luminescent response also was obtained in narcotized specimens by increasing the number of stimuli. This is ascribed to summation in the photogenic cells as the result of direct stimulation.

The temporal characteristics of the response curves and the rate of rise of light intensity have been critically examined. It is found that under a higher frequency of stimulation or more prolonged stimulation there is an increase in the rate of rise of light intensity, as well as an increment of light intensity, and an increase in the total amount of light. The rate of rise is directly related to the intensity of the response. The latent period is also reduced by increasing the period of stimulation. The argument is developed that the response curve reveals the course of oxidation of extruded photogenic material, that light appears subsequent to the series of events involved in photogenic secretion, and that the luminescent curve is only an indirect indication of the magnitude of the events preceding it. The rate of rise of light intensity and the length of latent period are indices of the velocity of the chemiluminescent reaction, which in turn is governed by the initial concentration of the photogenic material.

Besides peripheral augmentation of the luminescent response, a process of central facilitation has been discovered in the nerve cord. This is most apparent n the posterior region of the body, and manifests itself as an increase in the number of segments responding as the duration or frequency of stimulation s raised. The threshold frequency lies at about 5 per sec. In addition, luminescence spreads posteriorly with greater facility than anteriorly, indicating greater resistance to postero-anterior transmission.

The effects of certain drugs on the luminescent response have been nvestigated. Atropine and *d*-tubocurarine fail to abolish the luminescence evoked by electrical stimulation. Acetylcholine does not appear to affect the magnitude of the response due to electrical stimulation, whereas eserine produces an enhanced response in a minority of specimens. In conjunction with the positive effect of acetylcholine in inducing luminescence in *Chaetopterus*, the augmentative effect of eserine provides suggestive evidence of a transmitter role for acetylcholine.

Histological examination of the photogenic glands has yielded inconclusive esults. The epidermis is supplied with abundant nerves lying beneath the photogenic cells, and these fibres are well positioned to mediate secretion. The photogenic cells are surrounded by thin protoplasmic layers which stain ike muscle, but lack birefringence which might be expected in fibrous protein. No myo-epithelial elements or muscle fibres were seen in the epidermis. The suggestion is made that the protoplasmic investment of the landular light cells is contractile, and expresses the cellular contents. A mechanism of this kind explains the physiological results obtained in the present investigation.

The implications of some of these results on the physiology of biouminescence and the nature of control in the normal life of the animal are discussed. In particular, it is pointed out that the luminous response of the liform notopodia is a triggered response which can be produced by a single mpulse. The spread of the response-area in the posterior region under ncreased stimulation raises the possibility of normal control of luminescence n that region of the body by alterations in the frequency and number of nerve mpulses.

ADDENDUM

Since sending this paper to press I have had the opportunity of reading a paper by Hasama (1941) which describes experiments he has carried out on luminescence in *Chaetopterus variopedatus*. After faradic stimulation Hasama notes that the luminescent response lasts 10–40 sec. and that the duration of luminescence remains constant and independent of the duration of stimulation when the latter exceeds a certain time. No data are offered to support this ough generalization, which is merely a roundabout way of describing fatigue. With the use of non-polarizable electrodes this author has recorded potential changes on the surface of the light-producing regions during luminescence. The resultant monophasic potentials appear to correspond with the luminescent response and are separable from muscle action potentials. Hasama believes that these potentials are electrical manifestations of the chemiluminescent reaction. Two kinds of cells are described in the epidermis of luminescent regions, viz. type 1 cells which contain granules staining well with toluidine blue, and poorly with eosin; and type 2 cells which stain with eosin and possess cilia. Hasama claims the first type are characteristic of luminescent regions, and are the photocytes, but for reasons which I have presented in earlier papers I believe that the photocytes are peculiar granular eosinophilic cells, lacking cilia. These cells are completely overlooked by Hasama. Harvey's recent book (1952) appeared too late to be consulted in the present work.

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NVESTIGATIONS ON THE MICROFAUNA NHABITING SEAWEEDS ON ROCKY COASTS

(UNTERSUCHUNGEN ÜBER DIE ALGENBEWOHNENDE MIKROFAUNA MARINER HARTBÖDEN)

. STUDIES ON THE VERTICAL DISTRIBUTION OF THE FAUNA INHABITING SEAWEEDS BELOW THE PLYMOUTH LABORATORY

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(Text-figs. 1–4)

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INTRODUCTION, MATERIAL AND METHODS

e present study forms part of a more extensive one on the ecological factors ich govern the distribution of the microfauna inhabiting seaweeds on ky sea-coasts. This part of marine ecology is lagging far behind other nches. I wish to stress two points in particular: first, that our understanding he composition of the littoral (intertidal) fauna would increase very much his fauna can be linked with the true infralittoral fauna, and, secondly, blems involving the synecological aspects of marine biology cannot be roached before more is known about the autecology of the animals nposing the cryptofauna (i.e. those living in the shelter of algae) and the causal factors determining their occurrence and distribution within a given area under stated conditions. On this point the work of T. A. Stephenson is especially relevant: as a result of his extensive investigations on the composition of the intertidal flora and macrofauna he has come to stress the importance of autecological rather than of biocenological work on the shore (Stephenson *et al.* 1942; Stephenson, T. A. & A., 1949). Moreover, he has clarified the intertidal terminology and introduced the most useful terms 'supra-' and 'infralittoral fringe'. Since his terminology appears to be the best suited to meet the needs of ecologists, I hope it will be generally applied in future. Most work on littoral ecology has been limited to macro-organisms but valuable data on the ecology of microfaunas of seaweeds have been supplied by Colman (1940), Kitching, Macan & Gilson (1934), and Dah (1948). Some ideas on the causes affecting the composition of faunas have been brought forward by Remane (1933, 1940), but with main reference to the bottom fauna. Such 'causal analysis', applied to the microfauna of algae attached to the bottom, is a primary aim of the present study. Dahl (1948 has called attention to the importance of the quantity of detritus, but otherwise almost nothing has previously been attempted in this line.

To establish a numerical basis for comparisons between intertidal and infralittoral samples, a uniform collecting technique is essential. On a rock substratum the only useful method seems to be that of diving. The sam conclusion was reached by Kitching *et al.* (1934), who used a diving-helmet t examine a sublittoral gully in Wembury Bay. Since it was my intentio simply to study the population of single tufts of weed, it was possible to carr out the sampling under water (infralittoral and littoral during high wate using nothing but glasses (made by Draeger, Lübeck), flippers (made b Pirelli, Milano), and a belt with pieces of lead fixed to it. The sampling coul be carried out only when the sea was fairly calm. A one-litre jar was carrie and placed carefully over the clump of weed to be examined, which, when th whole was inside the jar, was torn off at the base as quickly as possible, th hand following and closing the opening of the jar immediately. Since a animals living in seaweeds have a tendency to fasten rather than to loose their hold if their habitat is disturbed (reaction to wave-shock?), it is like that my samples contained almost the whole population of the clump. Th alga was carefully shaken in formalin, branch by branch, and the derive material examined under a dissecting microscope. All animals were picke out and counted. The nematodes, which were collected for special stud were transferred to glycerin-jelly and mounted on slides for examin tion.

The whole material was collected just below the Plymouth Marine Biologic Laboratory, on the rocks adjoining the men's bathing place (Tinside). T limestone rocks here are broken and fissured, and supply habitats of varyi degrees of shelter. For a description of the area see Evans (1947*b*, p. 17

nside to West Hoe). It is only necessary to add that the deposition of diment is rather heavy along this piece of shore, with important effects. ave-action being fairly strong the sediment is distributed unevenly, being cumulated in dense tufts of weeds like *Gelidium corneum*, while tall and rub-like weeds as *Fucus serratus* may be almost free of silt.

The Rivers Tamar and Plym discharge into Plymouth Sound and cause variable reduction in the surface salinity of the sea water. This effect, wever, is large only in winter. Fluctuations in the volume of the more ratic Plym particularly affect Tinside. The silt-laden surface skin of fresh ater penetrating far into the Sound, after spells of heavy rain, is a familiar ght from the Laboratory.

No continuous measurements of salinity have been made at Tinside itself, ut a fairly accurate picture can be obtained by extrapolating from Milne's ta (1938). The salinity values for Tinside can be taken as identical with ose for Drake's Island, or somewhat less during wet periods. It is evident at in winter quite large falls in salinity may occur, and appreciable differences ily between high and low water. However, the present work did not start ntil after 2 or 3 months of settled summer conditions and the winter can be nored.

From April onwards it can be said that high-water salinities are normally tween 32 and 34 ‰ (Typically 33 ‰), and low-water salinities between and 32·5 ‰. The daily (or rather 12 hr.) fluctuation is normally less than ‰. After 2 or 3 days of heavy rainfall inland a temporary fall below 30 ‰ ay occur at low tide, but is not likely to have occurred in the summer of 50 up to the time the work had been completed.

The conditions at Tinside, therefore, as applied to this study, can be garded as truly marine with some polyhaline influences (see Dahl, 1948). his view is, moreover, supported by the character of the fauna, which is rely marine without any brackish component.

As regards the relation of shore organisms to tidal levels in the Plymouth ea, reference is made to Colman (1933), Moore (1935), Evans (1947*b*), etc., ho were dealing primarily with the macrofauna and flora. Certain heights d ranges are taken from these authors.

All tidal levels here are given in metres and are referred to *Chart Datum* for e Plymouth area (see The Admiralty Tide Tables). The position of some of e standard levels (see Hartley & Spooner, 1938) is as follows:

E.H.W.S.T.	+5·10	E.L.W.N.T.	+1·78
M.H.W.S.T.	4·78	M.L.W.N.T.	1·40
M.H.W.	4·26	M.L.W.	0·75
M.H.W.N.T.	3·73	M.L.W.S.T.	+0·06
E.H.W.N.T.	3·24	E.L.W.S.T.	−0·47
M.T.L.	2·49		

The seaweeds investigated in this work were collected in the following ranges (which should not be taken as the actual distribution limits):

Ceramium sp.	+2·75 to +0·80
Lomentaria articulata	+1·90 to +1·75
Fucus serratus	+2·00 to +0·20
Porphyra laciniata	+1·95 to +1·50
Gigartina stellata	+2·00 to +1·00
Cladophora rupestris	+3·25 to +2·50
Gelidium corneum	+2·75 to +1·10
Nitophyllum punctatum	−0·70 to −3·00

The material was obtained during a stay at the Plymouth Laborator between 14 July and 17 August 1950. I wish to thank all members of th Laboratory's staff, above all Mr F. S. Russell, F.R.S., not only for thei readiness to help and for all sorts of advice, but also for the spirit prevailin at Plymouth which makes work so easy and the Laboratory itself the mos excellent of its kind in Europe. My visit was made possible by financia support from several private institutions and the 'Bundesministerium fü Unterricht', at Vienna.

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General Remarks on the Vertical Distribution of the Fauna of Intertidal Seaweeds

The vertical distribution of littoral animals and plants has been studied bes in macro-organisms. The zonation of seaweeds, barnacles, limpets, peri winkles, etc., and thus the degree of exposure to air and wave-action whic they can endure, is more or less directly observable on the shore. This is du not only to the size of the organisms, but also to the fact that the rocks whic serve as a substratum for them may not change appreciably in compositio and texture down the length of the shore. The influence of the substratu on the distribution of these organisms may be almost negligible, and th vertical gradients of air-exposure and surf-action reveal themselves clearly i the well-defined zoning (or distribution limits) of the animals and plants i question.

The situation is quite different for micro-organisms inhabiting seaweed he different species of algae serving as substratum for the microfauna show ch different features in height, shape and consistency that exposure and ave-action, even within a very restricted area, by no means act uniformly on eir inhabitants. It is obvious that the animals inhabiting a dense tuft of *elidium corneum* or *Lichina pygmaea* are much more sheltered against siccation or wave-shock than those living on *Fucus serratus* or *Ascophyllum* *dosum*. Therefore, if we suppose that both types of plants might extend over e same vertical range on a rocky coast, the upper level of some littoral imals might well be much higher in *Gelidium* and *Lichina* than in *Fucus* and *scophyllum*. Hence it follows that the vertical distribution of the microfauna uld be studied best within one single kind of seaweed extending over sufficiently large vertical range. Numbers of animals per weight or area-it of the plant from different levels would then be directly comparable.

It is, however, not very often that the conditions are suitable for this kind of vestigation in the littoral and upper infralittoral: even within a very limited ea of the seashore there is normally a variety of algae some of them forming ry narrow belts. To get a fairly true and complete picture of the vertical stribution of the microfauna in the whole area under consideration, the pulations of different seaweeds must be studied together. A direct imerical comparison between samples from different weeds is hardly ssible owing to their great differences in structure. The best that can be ne is to express the vertical distribution of the microfauna in terms of *erage 'dominance-values'* (see p. 158) from as many and as different samples possible. This procedure, it should be remembered, cuts down the fferences between single populations and gives an average picture only of vertical distribution, the reliability of which can be improved by increasing e number of samples.

Different organisms are subjected in a different way to the factors of tidal posure. The prototypes of littoral zonation: seaweeds, barnacles, peri-nkles, etc., are either sessile, hemi-sessile or very slowly moving organisms. hey cannot counteract rapid changes of environmental conditions by oving about. We have to presume, therefore, that the animals must be able endure any condition to be expected in the inhabited area (as proved, for ample, by Jacubowa & Malm, 1931, for several bottom-animals, in their ility to withstand anaerobic conditions). In the littoral area the habitat of ese animals is either a well-defined 'zone' or has at least one well-defined per or lower limit. The same conditions should apply to members of the crofauna which are truly sessile, e.g. Bryozoa and Hydrozoa, or hemi-ssile like the tubicolous polychaetes, amphipods (*Corophium*) and isopods *anais cavolinii*); or, indeed, to all animals with slow powers of movement. he latter, it is true, might find shelter in the denser parts of the algae they habit, or in minute crevices of the substratum filled with sediments in-cessible to bigger animals, and so possess the ability of counterbalancing the challenge of the environment if this becomes too unfavourable, but th movements are too small to effect the average vertical distribution on th shore. A great deal of the microfauna belongs to this class, e.g. nematode ostracods, small molluscs like *Rissoa* and *Lasaea*, halacarids, etc. There ar other animals which would be able to swim or to crawl quickly and to chang place during, say, the rise and fall of the tide, but which do not in fact do s since they are hardy enough to stand any change in the environmenta conditions. In this respect they behave like hemi-sessiles. Among them ma be included several amphipods, especially *Hyale* spp., which are well-adapte inhabitants of the littoral seaweeds on all rocky coasts.

Finally, there is a group of animals not only able to move relatively far an fast, but also using this ability, i.e. compensating for changes in the environmental conditions by active motion over comparatively great distances. Man of the harpacticids and some amphipods like *Stenothoë monoculoides* seem t belong to this group. The study of their average vertical distribution does n give a true picture of their actual distributions, which might be quite differen under different conditions, for example during high and low tide.

RESULTS OF THE SURVEY AT TINSIDE

The Population Counts from Different Algae

Gelidium corneum

For investigating the vertical distribution of the microfauna inhabiti a single species of alga in the Tinside area no better prototype could be chos than *G. corneum*. Not only does it extend over a sufficiently large vertic range of about 1·65 m., but also, due to its dense, tuft-like shape, it contai an almost incredibly large fauna, whereby the numerical comparison different samples is facilitated.

The results are given in Table I. The numbers of specimens are referr to 1 g. of living alga weighed in dry condition after pressing between piec of cloth. The samples are arranged from left to right in descending sequen of tidal level. As was to be expected, the number of specimens in the differe samples varies very much, from 343 in G-14 to 2818 in G-4. This variati is due in a very slight degree only to differences in the vertical position the sample. In my opinion the most important factor is the silt content. attempt was made to estimate the amount of silt by recognizing five arbitra classes, ranging from 0 to 4. Plotting number of specimens against the classes brought out an evident correlation, at least for nematodes, sabell and oligochaetes. This agrees with Dahl (1948). This interesting subject however, beyond the scope of the present paper. It is treated, at least as far the nematodes are concerned, in my previous papers (Wieser, 1951, 1952)

In spite of fluctuating values, the distribution of several species through the range of the zone shows an obvious vertical gradient. For that purpos

Sediment-classes ...	3	0	2	2	3	3	1	3	1	3	3	1	1	1	3	2	4
Weight (g.) ...	1·3	2·2	1·2	1·0	0·8	1·4	1·3	1·2	1·4	1·3	1·6	2·0	0·6	0·6	1·6	1·2	0·8
Nematoda	1270	215	665	600	964	657	470	638	286	658	700	172	373	103	487	370	835
Copepoda	140	48	23	26	180	19	55	65	30	140	113	72	171	51	185	12	14
Ostracoda	130	25	17	32	280	21	41	30	88	119	87	23	40	45	103	7	19
Amphipoda:																	
Hyale nilssoni	5	25	6	18	10	2	10	.	1	.	1	1	.	.	.	2	26
Corophium spp.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	12	9
Stenothoë monoculoides	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	35	18
Polychaeta:																	
Fabricia sabella	38	.	29	26	614	311	217	483	4	548	420	142	375	40	270	150	44
Amphiglena mediterranea	.	.	.	.	.	.	.	.	1	.	.	.	.	1	.	5	.
Oridia armandi	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.
Grubea pusilla	.	.	.	.	125	8	2	3	.	4	3	1	.	.	3	6	.
Syllis armillaris	.	.	.	.	.	.	3	.	.	.	.	1	.	.	.	.	.
Polydora hoplura	.	.	.	.	.	.	.	2	.	.	.	.	.	.	3	3	.
Exogone gemmifera	.	.	.	.	.	.	.	1	.	.	.	.	.	.	2	.	.
Cirratulus cirratus	.	.	.	.	.	.	.	1	.	.	.	.	.	.	6	.	.
Aonides oxycephala	.	.	.	.	.	.	.	.	.	.	.	.	1	.	2	.	.
Odontosyllis ctenosoma	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.
Capitellides giardi	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
Oligochaeta	225	130	.	12	230	258	77	64	70	292	172	32	139	2	50	89	90
Halacarida	14	7	13	28	14	6	5	20	6	21	43	4	21	12	3	62	227
Hyadesia sp.	.	1	.	3	3	.	10	2	.	.	1	.	.	.	.	.	.
Chironomidae (larvae)	58	51	70	114	129	24	48	60	23	100	54	40	75	50	45	10	9
Gastropoda:																	
Littorina obtusata	.	.	2	1	.	.	4	.	4	.	.	.	.	.	.	1	3
Skeneopsis planorbis	3	.	14	83	14	5	7	10	3	.	30	5	21	.	1	50	83
Cingulus cingillus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
Rissoa parva	.	.	.	4	.	.	2	.	.	.	.	.	.	.	.	.	.
Indet.	2	.	1	3	.	.	.	.	.	36	.	.	6	25	.	.	.
Pelecypoda:																	
Lasaea rubra	685	265	348	1864	164	371	120	20	78	3	2	.	5	9	6	50	431
Mytilus edulis	16	3	10	4	5	10	2	5	3	1	2	3	5	2	3	8	12
Isopoda + Anisopoda:																	
Idothea neglecta ? + *granulosa*	.	.	.	.	.	.	.	.	.	.	.	.	11	.	.	5	10
Jaera marina	.	3	.	.	.	2	1	.	.	.	.	.	.	.	.	.	.
Naesa bidentata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.
Munna sp.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
Tanais cavolinii	32	2	.	.	4	3	1	.	.	.	23	.	.	3	.	7	23
Pantopoda:																	
Phoxichilidium femoratum	.	.	.	.	1	.	1	1	.	.	.	.	.	.	.	.	.
Total	2618	775	1208	2818	2737	1697	1079	1409	597	1925	1651	497	1243	343	1173	885	1853

is convenient to combine several samples and to consider their average number of specimens.

The most interesting change is the decline in number of *Fabricia sabella* between +2·0 and +2·5 m. Up to +2·0 m. the average number of specimens is 263 per g. dried weed, while it drops to 25 in the four uppermost samples at +2·50 and +2·75 m. It appears, therefore, that a 'critical level' for the species may exist between +2·00 and +2·50 m. This level may be taken and tested with respect to other species or groups. In Table II are given the average values for stations G-1 to G-4 compared with those for G-5 to G-17. Three of the more numerous types are selected—*F. sabella*, Oligochaeta and *Lasaea rubra*. Each deserves comment.

(i) *Fabricia sabella*. I do not hesitate to call the level between +2·0 and +2·5 m. a true 'critical level' (in the sense of Colman and Evans) in the Tinside area for this species. The bulk of the population does not extend beyond this level (which does not preclude the penetration of some specimens into higher zones). It is difficult to compare my figures with those of Colman (1940) from Wembury Bay, since that area shows some important ecological

TABLE II. AVERAGE POPULATION DENSITY PER G. OF DRIED WEED

Height (m.)	... +2·75 to +2·50 m.	+2·00 to +1·10
Fabricia sabella	25	263
Oligochaeta	94	117
Lasaea rubra	790	97

differences from Tinside. Colman reports a few *F. sabella* (which also a Wembury penetrates farther upshore than any other polychaete) from *Fucu spiralis* about 1 m. higher up than the upper level of my *Gelidium corneum* and a great number of specimens from a single sample of *Ascophyllum nodosum*+*Polysiphonia lanosa*, about +3·30 m. Then it is not until the *Laminaria* holdfasts that *Fabricia sabella* makes its appearance again in grea numbers (this habitat being the lowest and most sheltered in Colman's samples).

(ii) *Oligochaeta*. The vertical gradient has apparently no significant influence on the distribution of this (or possibly two) species. These animals show a strong affinity to rich silt-content, and vertical differences could not be detected with certainty in the seventeen *Gelidium* samples. In Colman's tables, also, the oligochaetes (*Lumbricillus pumilus* and *L. scoticus*) show a somewhat irregular distribution with no apparent correlation with tida exposure.

(iii) *Lasaea rubra*. The difference in numbers between the four higher and the thirteen lower samples is more striking than in any of the species investigated. It should, however, be noted that the lowest samples (G-17), whose content of sediments was extremely high, also contained an unexpectedly high number of *Lasaea*. My figures agree fairly well with Colman's statement to the ffect that his four samples of the lichen *Lichina pygmaea* (between +2·68 and +4·13 m.) contained almost incredible numbers of *Lasaea rubra*, while the pecies did not occur farther down. The relation between the shape of the eeds and their populations (Wieser, 1951) should also be kept in mind: *elidium corneum* and *Lichina pygmaea* are very alike in shape and structure, nd both may harbour large populations of this lamellibranch for the same eason. (Colman has found an average of about 945 specimens per 1 g. damp *ichina*, while I got about the same number in my four highest *Gelidium* amples but per 1 g. of dried weed: thus, Colman's figures are still higher; lthough, if we remember that in my *Gelidium* samples the numbers of ematodes, polychaetes, oligochaetes, copepods and ostracods were often igher and sometimes not very much lower than those of *Lasaea rubra*, while n Colman's samples *Lasaea* is absolutely dominant, the *Gelidium* of Tinside roves to be still richer in living organisms than the *Lichina* of Wembury Bay. s a matter of fact, the number of organisms in *Gelidium corneum* is comparable ith those of rich soils which—according to Franz (1950) and not to the uthors mentioned by Colman—often reach several million specimens er m.^{2}).

There are several other types which might illustrate the three possible elations to the factor of tidal exposure, viz. (i) the decline in number down- hore, (ii) the decline in number upshore, and (iii) the more or less even istribution. Thus to the first belong *Hyale nilssoni* (although low down at G-17 fairly large number was found), *Hyadesia* sp., probably *Jaera marina* and he chironomid larvae (*Clunio marinus* + *Trichocladius* cfr. *vitripennis*, not eparated). For the latter (average of seventy-eight specimens above, fifty- ne below +2·0 m.) there may be a critical level at about the lower limit of *elidium corneum*, which also accords with Colman, who did not find any nsect larvae below +1·20 m. It should be mentioned that on all seashores hich I have had the opportunity to investigate I have always found chiro- omid larvae among those animals which could be regarded as typical nhabitants of the higher intertidal zones.

To the second group belong *Corophium* spp., *Stenothoë monoculoides*, and ll Polychaeta errantia (none of which passes above the +2 m. level). To the hird group belong *Mytilus edulis*, most of the common nematodes like *nticoma limalis*, *Thoracostoma figuratum*, *Enoplus communis* (the dominant pecies in nearly all samples), *Dolicholaimus marioni*, *Halichoanolaimus obustus*, *Monoposthia costata*, *Chromadora nudicapitata*, etc. (see Wieser, 951); and probably *Tanais cavolinii* and the two Halacarida (most probably *hombognathus pascens* and *R. seahami*).

he Leaf-Like Algae Porphyra laciniata *and* Nitophyllum punctatum

It might be permissible to study numerically the microfauna not only ithin one species of seaweed but also within a single morphological type.

If it is agreed that it is the shape and consistency of the weed that is of prime importance to the composition of its fauna, two different species of algae of similar shape can be treated as a single species. At any rate in the very simple case of two seaweeds with flattened, uniform thalli like *Porphyra laciniata* and *Nitophyllum punctatum* there can hardly be any objection to this method. In the Tinside area *Porphyra* extends to about +2·0 m., while *Nitophyllum* is a typical infralittoral species which I have collected down to −3·0 m. The vertical distribution of the microfauna can thus be examined over a range of about 5 m. on a more or less comparable substratum. What is still more important to this study is that the change in the fauna which take place between the intertidal and infralittoral zones can now be investigate Altogether, I obtained three samples of *Porphyra laciniata* between +1·5 and +1·95 m. and four samples of *Nitophyllum punctatum* between −0·7 and −3·00 m. The results are given in Tables III and IV. For the nematode in Table III, I have to confine myself to dominance-values, since I was unabl to study the whole collection. Therefore only a rough picture of the com position of the fauna is given. The three samples of *Porphyra* are pooled.

TABLE III. DOMINANCE-VALUES OF NEMATODES IN THREE SAMPLES OF *PORPHYRA LACINIATA* AND FOUR SAMPLES OF *NITOPHYLLUM PUNCTATUM*

(*P.* = *Porphyra*; *N.* = *Nitophyllum*.)

No. of sample ...	L-1 to 3	L-4	L-5	L-6	L-7
Height (m.) ...	+1·50−1·95	−0·70	−1·20	−1·20	−3·00
Alga ...	*P.*	*N.*	*N.*	*N.*	*N.*
Anticoma limalis	.	.	.	.	5
Enoplus communis	16	9	4	18	15
Dolicholaimus marioni	5	.	.	.	.
Oncholaimellus diodon	.	.	.	.	8
Oncholaimus dujardini	5	.	.	.	.
Total Enoplidae	26	9	4	18	28
Cyatholaimus demani	5	5·5	6	4	4
Desmodora serpentulus	.	.	.	4	.
Monoposthia costata	5	.	2	.	.
Parasabatiera similis	.	.	2	.	.
Total Cyatholaimidae + Desmodoridae	10	5·5	10	8	4
Spilophorella paradoxa	.	2	.	.	.
Euchromadora tridentata	5	3·5	2	4	4
Hypodontolaimus inaequalis	.	.	2	.	.
Prochromadorella paramucrodonta	.	7	2	22	.
P. neapolitana	.	2	4	.	.
Neochromadora poecilosomoides	.	16	26	22	33
Chromadora nudicapitata	32	18	2	7	8
C. brevipapillata	.	29	47	15	19
C. macrolaima	10	.	.	.	.
Heterochromadora germanica	16	.	.	.	.
Prochromadora longitubus	.	2	.	.	.
Total Chromadoridae	63	79·5	84	70	64
Theristus setosus	.	5·5	.	4	4
Total Monhysteridae	.	5·5	.	4	4
No. of specimens examined	19	55	47	27	27

ABLE IV. NUMBER OF SPECIMENS PER 50 G. OF DRIED WEED IN THREE SAMPLES OF *PORPHYRA LACINIATA* AND FOUR SAMPLES OF *NITOPHYLLUM PUNCTATUM*

(+ = common; + + = very common. *P.* = *Porphyra*; *N.* = *Nitophyllum*.)

	Littoral			Infralittoral			
No. of sample ...	L-1	L-2	L-3	L-4	L-5	L-6	L-7
Height (m.) ...	+1·95	1·50	1·50	−0·70	−1·20	−1·20	−3·00
Alga ...	*P.*	*P.*	*P.*	*N.*	*N.*	*N.*	*N.*
Weight (g.)	35	22	16	3·4	9	5	2·9
Nematoda (see Table III)	23	22	10	2130	462	380	544
Copepoda	4	90	95	1605	885	490	1071
Ostracoda	.	.	.	60	38	50	34
Amphipoda:							
Hyale nilssoni	2	2	.	.	.		.
Jassa falcata	.	2	6	15	15		136
Stenothoë monoculoides	.	.	3	.	.		.
Aora typica	.	.	.	60	112		.
Nannonyx goesi	.	.	.	30	.		.
Leucothoë spinicarpa	.	.	.	15	.		.
Corophium spp.	.	.	.	1020	455		102
Apherusa bispinosa	.	.	.	.	28		.
Polychaeta:							
Amphiglena mediterranea	.	4	.	.	.		.
Oridia armandi	.	.	.	45	.		17
Platynereis dumerilii	.	4	3	840	70		34
Lagisca extenuata	.	.	.	75	.		17
Odontosyllis ctenosoma	.	.	.	330	21		.
Exogone gemmifera	.	.	.	60	7		34
Grubea clavata	.	.	.	75	7		.
Autolytus aurantiacus	.	.	.	.	21		.
Pterosyllis formosa	.	.	.	.	7		.
Sphaerosyllis hystrix	.	.	.	.	7		.
Halacarida	.	2	3	.	126	30	34
Chironomida (larvae)	7	2	3	.	.	.	.
Gastropoda:							
Littorina obtusata	.	4	3	.	5	.	.
Rissoa parva	.	2	6	180	38	80	51
Tricolia pullus	.	.	.	.	11	30	.
Pelecypoda:							
Lasaea rubra	2	.	.	.	.	.	.
Mytilus edulis	.	.	.	60	5	.	.
Isopoda:							
Idothea neglecta ? + *granulosa*	18	12	.	15	.	.	.
Munna sp.	.	.	.	15	.	.	.
Bryozoa:							
Membranipora membranata	.	.	.	+ +	.	.	.
Tunicata:							
Botryllus schlosseri	.	.	.	+ +	+	+	+
Total no. of specimens	56	146	132	6630	3400		2074

In Table IV the number of specimens is given per 50 g. *of dried weed*. Sample L-4 was completely overgrown with *Membranipora membranata* and *Botryllus schlosseri*, and was extremely rich in specimens. The amphipods and polychaetes in samples L-5 and L-6 had to be treated together.

These two tables seem to show some significant contrasts between the littoral (intertidal) and infralittoral zones. Foremost, the increase in total population, in the descent across the low-water level, is most striking. The average number of specimens in the three *Porphyra* samples is 111 per 50 g. as against 3026 in the four *Nitophyllum* samples. Likewise I found twenty species above as against forty species below C.D. (excluding copepods, ostracods and halacarids). Some of the species found in the *Nitophyllum* samples do not reach a maximum until the infralittoral zone is reached (although some of them might well extend farther upshore in denser weeds where they are better sheltered from desiccation—see p. 149 above). Amongst the nematodes are *Neochromadora poecilosomoides*, *Prochromadorella paramucrodonta* and *Chromadora brevipapillata*. Most of the polychaetes, as for example *Lagisca extenuata*, *Exogone gemmifera* and *Grubea clavata*, as well as the tunicate *Botryllus schlosseri*, and the snail *Tricolia pullus* appear to belong to this group. Colman's data agree with respect to polychaetes and tunicates, since it is only in his lowest and most sheltered samples of the *Laminaria* holdfasts that the Polychaeta errantia suddenly occur, being exceedingly rich here both in specimens and species (amongst them *Lagisca extenuata* and *Exogone gemmifera*). The tunicates similarly are strictly confined to the *Laminaria* holdfasts. Comparably, with regard to the true littoral species, viz. *Hyale nilssoni*, the chironomid larvae, and *Lasaea rubra*, the conclusions drawn from the *Gelidium corneum* samples find further support from those of the leaf-like algae (or, if the data seem too scanty to be confirmative, at least they do not contradict them). *Hyale nilssoni*, as well as *Lasaea rubra* and the insect larvae in Colman's samples, are confined to the upper part of the littoral region.

It is no doubt primarily due to the shape of the leaf-like weeds that the difference in the littoral and infralittoral populations, and analogously the difference in the littoral and infralittoral ecological conditions, can be so clearly demonstrated. The flattened thalli of these weeds offer almost no protection to their inhabitants. It is thus natural that they are deprived of animals in zones where the degree of tidal exposure is great, while as soon as the environmental conditions become more favourable (as in the infralittoral zone and especially beneath the protective canopy of tall algae like *Laminaria*) life reappears in the abundance typically associated with surfaces below the level of the sea.

The Tufted Algae: Ceramium *sp.*, Cladophora rupestris *and* Lomentaria articulata

These three weeds occupy much of the tidal zone. Altogether twenty samples were collected ranging from +0·8 to +3·25 m. The results are given in Table V. The three algae differ somewhat in their structure, but as a whole I think their populations can be compared numerically without

(N.B. Samples T-12, 13 and 14 held a particularly large quantity of sediment.)

No. of sample ...	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12	T-13	T-14	T-15	T-16	T-17	T-18	T-19	T-20
Height (m.)	+3·25	3·25	2·75	2·60	2·50	2·50	2·50	2·50	2·50	2·50	2·25	2·00	2·00	1·90	1·75	1·60	1·60	1·40	1·40	0·80
Seaweed	*Cl.*	*Cl.*	*Ce.*	*Cl.*	*Ce.*	*Cl.*	*Ce., Cl.*	*Cl.*	*Cl.*	*Cl., Ce.*	*Ce.*	*Ce.*	*Ce.*	*Lom.*	*Lom.*	*Ce.*	*Ce.*	*Ce.*	*Ce.*	*Ce.*
Weight (g.)	0·9	0·9	0·5	1·8	1·0	2·0	0·8	0·15	1·2	0·4	2·0	0·5	0·5	2·0	3·6	2·0	1·7	3·5	2·6	1·7
Nematoda	91	84	20	17	120	53	172	28	4	55	93	1400	1096	31	249	450	40	71	116	70
Copepoda	27	10	34	34	16	9	29	7	21	42	3	86	48	4	4	27	4	105	3	20
Ostracoda	10	14	44	17	32	11	20	13	9	67	.	88	92	15	3	6	2	1	1	.
Amphipoda:																				
Hyale nilssoni	28	25	2	14	34	20	33	28	12	25	.	8	.	3	2	.	.	1	.	.
Stenothoë monoculoides	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
Corophium spp.	.	.	.	.	.	.	.	.	.	.	.	.	6	.	.	.	.	1	.	3
Jassa falcata	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
Polychaeta:																				
Fabricia sabella	1	2	.	.	.	1	.	.	.	2	.	172	168	13	.	11	.	1	.	2
Oridia armandi	.	.	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.
Grubea pusilla	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	3	.	.	.	.
Odontosyllis ctenosoma	.	.	.	.	.	.	.	.	.	.	.	4	.	.	1	.	.	.	.	.
Amphiglena mediterranea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	5	.	.	.	.
Oligochaeta	15	25	.	.	3	.	.	.	.	.	1	116	18	3	2	.	.	.	1	.
Halacarida	14	17	.	2	3	2	7	7	2	7	.	68	10	4	15	2	.	2	.	2
Hyadesia sp.	.	.	.	.	1	.	.	.	.	.	1	.	.	3	9	2	5	1	.	.
Chironomida (larvae)	25	27	10	35	34	31	85	14	2	152	4	28	22	11	4	27	12	6	3	4
Gastropoda:																				
Skeneopsis planorbis	.	.	.	.	3	1	.	7	.	.	.	182	.	25	1	.	2	1	.	.
Rissoa parva	.	.	.	.	.	.	.	.	.	.	1	10	.	.	.	.	1	3	.	1
Littorina obtusata	.	.	.	2	1	1	.	6	.	.	.	6	.	3	.	.	.	3	.	.
Cingulus cingillus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.
Indet.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	8	23	.	1	.	.
Pelecypoda:																				
Lasaea rubra	261	239	42	8	4	83	325	56	2	20	.	234	.	1	14	62	.	1	.	.
Mytilus edulis	.	.	.	.	.	.	.	.	.	.	.	12	6	1	3	3	.	.	.	.
Isopoda + Anisopoda:																				
Idothea neglecta ? + *granulosa*	.	.	.	14	1	3	4	13	3	5	3	.	16	2	2	2	3	6	1	8
Jaera marina	2	.	2	.	1	1	.	.	.	.	.	8	.	.	.	.	.	.	.	.
Naesa bidentata	.	.	.	.	.	.	.	.	.	.	1	.	.	.	2	.	.	.	.	4
Tanais cavolinii	.	.	.	2	.	1	.	.	.	2	.	.	.	.	.	.	.	.	.	.
Total	474	443	154	145	253	217	676	179	55	377	107	2428	1482	119	312	623	68	205	125	116

important adjustments for the differences between them. The results agree satisfactorily with those obtained from the other algae, and only brief comment is necessary.

Decline in number downshore. The distribution of *Hyale nilssoni* agrees particularly well with what has been said above. The average lower level seems to lie at about +2·0 to +2·5 m. with some allowance, of course, for individual irregularities and special ecological conditions. In the tufted algae there is an average of 22 specimens per 1 g. dried weed above, against 1·4 below +2·5 m. Again, the distribution of *Lasaea rubra* agrees fairly well. The bulk of the numbers is at any rate to be found above +2·0 m. The samples T-1 and T-2 (+3·25 m.) with their average number of about 250 per g. almost approach the centre of the area which according to Colman is occupied by *L. rubra* in Wembury Bay—i.e. between +2·68 and +4·13 m. For the chironomid larvae *Clunio marinus* and *Trichocladius* cfr. *vitripennis* the results derived from *Gelidium corneum* (p. 153) also apply here: the lower level seems to be situated at about +1·10 m., which accounts for the rather slow decline in numbers in the present samples. There is, nevertheless, a marked difference between the samples above and those below 2·5 m. (41·5 against 12 specimens per g.).

Decline in number upshore. The 2 m. level proves again to be critical for all polychaetes. From Table IV it is suggested that *Rissoa parva* has the centre of its distribution decidedly lower than the lower level of the tufted algae, probably in the infralittoral zone (where it has been found, too, by Kitching *et al.* 1934), although single specimens can go up to about +2·50 m.

Even distribution. This appears to be shown by *Idothea neglecta* and the halacarids, the numbers of other species being too scarce for any conclusions to be drawn. Referring to data, as yet unpublished, I can add here most of the common nematodes, especially *Enoplus communis*, *Chromadorella parapoecilosoma*, *Heterochromadora germanica* and *Chromadora nudicapitata*.

'*Average*' *Distribution*

As already noted (p. 149), when data are presented of the fauna of seaweeds of very different structure, the numbers per unit weight tend to reflect the effect of the different substrata on the fauna rather than that of the vertica gradient. To overcome this difficulty it is usual to express the occurrence o the animals as a percentage of the total (giving so-called 'dominance values' and to compare as many samples as possible, aiming at an average picture o their vertical distribution. This procedure is followed in the present section in which the vertical zoning is examined of amphipods, polychaetes and nematodes, in the littoral and upper infralittoral zones, regardless of the differen algae from which they have been taken.[1]

---

[1] The data of this section are not only based on the samples dealt with in the previou chapter but of all those listed in the Appendix. It was, however, impossible to study ever specimen; only a fraction of each sample has been examined taxonomically, the number which is referred to in the tables of this section.

mphipoda

The vertical distribution of the ten species found (excluding *Corophium* p.) is given in Table VI. It is possible to compare these data with e distribution of the amphipods at Wembury combining Colman's and itching's results, from collections obtained between 1930 and 1932. Kitching otained his samples by diving to about 10 ft. below C.D.

ABLE VI. DISTRIBUTION OF AMPHIPODS IN THE TINSIDE AREA. THE FIGURES GIVE PERCENTAGE OCCURRENCE AT EACH LEVEL ('DOMINANCE VALUES')

Height (m.)	+3·50 to +2·75	+2·75 to +2·00	+2·00 to +1·50	+1·50 to 0	0 to −3·00
Hyale nilssoni	100	87·3	46·7	7·0	.
Apherusa jurinei	.	11·1	10·3	16·2	15·7
Jassa falcata	.	0·3	31·7	18·4	33·2
Stenothoë monoculoides	.	.	10·0	51·0	9·3
Gammarus locusta	.	.	1·3	.	.
Pleonexes gammaroides	.	.	.	7·7	.
Apherusa bispinosa	.	.	.	.	4·0
Aora typica	.	.	.	.	29·0
Nannonyx goesi	.	.	.	.	6·2
Leucothoë spinicarpa	.	.	.	.	3·1
No. of samples containing amphipods	4	8	20	13	4
No. of specimens	108	199	166	276	49

ABLE VII. DISTRIBUTION OF AMPHIPODS IN WEMBURY BAY, COMPUTED FROM COLMAN (1940) AND KITCHING *et al.* (1934). FIGURES GIVE PERCENTAGES OF TOTAL AMPHIPODA (DOMINANCE VALUES)

	Colman			Kitching
Height (m.)	+4·63 to +3·50	+3·50 to +1·50	+1·50 to about 0	about 0 to −3·30
Hyale nilssoni	100	97·3	8·7	.
Melita sp.	.	0·2	.	.
(?) *Marinogammarus obtusatus*	.	0·2	.	.
Amphithoë rubricata	.	1·7	0·5	.
Microjassa cumbrensis	.	.	3·0	.
Pleonexes gammaroides	.	.	1·6	.
Hyale pontica	.	.	0·6	.
Stenothoë monoculoides	.	.	0·5	.
Tritaeta gibbosa	.	.	0·3	.
Leucothoë incisa	.	.	0·3	.
Biancolina cuniculus	.	.	0·17	.
Jassa falcata	.	.	50·3	22·6
Apherusa jurinei	.	0·5	9·4	6·4
Elasmopus rapax	.	.	7·1	4·5
Microdeutopus damnoniensis	.	.	5·0	1·8
M. chelifer	.	.	1·0	
Podocerus variegatus	.	.	5·3	0·17
Eurystheus maculatus	.	.	4·0	1·3
Lembos websteri	.	.	2·3	1·0
No. of samples	13	14	17	11
No. of specimens	466	618	621	593

To make this comparison I computed the dominance values of the amphipods from Colman's tables and from Kitching's *Distomus-Halichondria* association. The Caprellidae were omitted, and from Kitching's tables only those species were extracted which were also represented in Colman's samples. I am fully aware of the objections which might be made to this combining of samples taken at different times and by different methods but, nevertheless, the results, set out in Table VII, are not discordant.

Owing to differences in the ecological conditions and positions of samples (algae extending farther upshore, no samples taken between +2·00 and +1·50 m.) the zones of height applied to Colman's data differ from those used for Tinside. Furthermore, the number of specimens and of species found at Wembury is much higher than that found at Tinside, mainly due to the extremely rich *Laminaria* holdfasts and to the fact that the lower zone in Wembury has been more thoroughly investigated. Nevertheless, it appears as if the distribution of *Hyale nilssoni*, *Apherusa jurinei* and the few *Pleonexes gammaroides* agrees fairly well in the two areas, although *Apherusa jurinei* is distinctly more common at Tinside than at Wembury. Kitching's *Jassa* '*dentex*' should be merged with *J. falcata* (see Sexton & Reid, 1951), so *J. falcata* is about as abundant in the infralittoral and lowest littoral zone in Wembury as at Tinside, though it reaches higher upshore in the latter area. The increase in number of species downshore seen in both areas is only to be expected for a marine group of animals.

TABLE VIII. DISTRIBUTION OF POLYCHAETES AT TINSIDE, AS IN TABLE VI

Height (m.)	+3·25 to +2·75	+2·75 to +2·00	+2·00 to +1·50	+1·50 to about 0	About 0 to −3·00
Fabricia sabella	100	100	61·2	32·4	.
Grubea pusilla	.	.	22·7	6·5	.
Amphiglena mediterranea	.	.	8·5	7·8	.
Syllis armillaris	.	.	1·9	0·5	.
Polydora hoplura	.	.	0·8	4·0	.
Cirratulus cirratus	.	.	0·3	1·8	.
Syllis krohni	.	.	0·5	.	.
Capitellides giardi	.	.	0·16	.	.
Odontosyllis ctenosoma	.	.	3·0	24·5	20·4
Platynereis dumerilii	.	.	0·8	13·2	56·1
Exogone gemmifera	.	.	0·8	0·7	6·0
Oridia armandi	.	.	0·3	5·6	2·0
Grubea clavata	.	.	.	1·8	5·1
Autolytus aurantiacus	.	.	.	1·3	2·6
A. prolifer	.	.	.	0·2	.
Spionidae sp.	.	.	.	0·5	.
Aonides oxycephala	.	.	.	0·2	.
Lagisca extenuata	.	.	.	.	5·1
Pterosyllis formosa	.	.	.	.	1·0
Sphaerosyllis hystrix	.	.	.	.	1·0
Phyllodocae maculata	.	.	.	.	1·0
No. of samples (only those with polychaetes)	1	5	26	19	4
No. of specimens	2	23	628	447	119

Species of the genus *Hyale* contribute to the fauna of the highest algal nes on most rocky seashores. For example, in the Mediterranean we find *ale prevosti* and *H. nilssoni* f. *stebbingi* to be the dominant amphipods for s 'biotope' and on the Chilean coast *H. hirtipalma*, *grandicornis* and one or o other species. (For this information I am indebted to Dr E. Dahl, who ndly permitted me to quote it from his yet unpublished material.)

TABLE IX. POLYCHAETA FROM WEMBURY. (SEE TABLE VII)

(+ = less than 0·1 %.)

	Colman			Kitching
Height (m.)	4·63 to 3·50	3·50 to 1·50	1·50 to about 0	about 0 to about 3·30
Fabricia sabella	100	98·3	54·2	4·4
Spirorbis borealis	.	1·5	7·0	2·8
Nereidae	.	0·2	See species	
Amphiglena mediterranea	.	.	10·6	.
Sphärosyllis erinaceus	.	.	6·1	.
Polydora giardi	.	.	6·0	.
Micromaldane ornithochaeta	.	.	5·8	.
Oridia armandi	.	.	4·3	.
Capitellides giardi	.	.	1·4	.
Polydora ciliata	.	.	0·6	.
P. caeca	.	.	0·4	.
Pholoë minuta	.	.	0·17	.
Exogone gemmifera	.	.	0·17	.
Odontosyllis ctenosoma	.	.	0·14	.
Dodecaria concharum	.	.	0·14	.
Sphaerosyllis ovigera	.	.	0·1	.
Eulalia bilineata	.	.	+	.
Eteone picta	.	.	+	.
Grubea limbata	.	.	+	.
Eusyllis lamelligera	.	.	+	.
Exogone brevipes	.	.	+	.
E. verrugera	.	.	+	.
Perinereis cultrifera	.	.	+	.
Polydora hoplura	.	.	+	.
Heterocirrus alatus	.	.	+	.
Polycirrus calientrum	.	.	+	.
Potamilla torelli	.	.	+	.
Jasmineira elegans	.	.	+	.
Hydroides norvegicus	.	.	+	.
Pomatoceros triqueter	.	.	0·5	22·0
Syllis gracilis	.	.	1·0	0·9
Trypanosyllis zebra	.	.	0·4	2·8
Platynereis dumerilii	.	.	0·17	12·5
Syllis variegata	.	.	0·17	1·4
S. armillaris	.	.	0·17	2·0
S. prolifera	.	.	0·1	0·6
Sabellaria spinulosa	.	.	+	4·4
Dasychone bombyx	.	.	+	1·2
Potamilla reniformis	.	.	+	0·6
Lagisca extenuata	.	.	+	0·3
Syllis ferruginea	.	.	+	0·3
No. of samples	2	6	13	11
No. of specimens	6	871	7975	358

TABLE X. NEMATODA FROM TINSIDE, FROM ALL SAMPLES EXAMINED (DOMINANCE VALUES)

(+ = less than 0·1 %.)

Height (m.)	+4·50 to 3·25	+3·25 to 1·50	+1·50 to 0	0 to −3·00
Leptosomatidae:				
Anticoma limalis	.	6·5	5·5	2·0
A. pellucida	.	0·5	+	.
Leptosomatum bacillatum	.	.	+	.
Thoracostoma figuratum	.	2·4	1·0	.
Th. (Pseudocella) trichodes	.	0·2	+	.
Oxystomatidae:				
Trefusia longicauda	.	0·3	.	.
Thalassoalaimus tardus	.	+	.	.
Halalaimus gracilis	.	+	.	.
Phanodermatidae:				
Phanoderma albidum	.	+	0·3	.
Enoplidae:				
Enoplus communis	62·0	30·0	36·4	13·0
Dorylaimidae:				
Dolicholaimus marioni	.	3·8	0·4	.
Syringolaimus striaticaudatus	.	+	+	.
Oncholaimidae:				
Krampia acropora	.	0·1	0·3	.
Cavilaimus macramphis	.	.	0·1	.
Pontonema vulgaris	.	+	.	.
P. donsi	.	+	0·15	.
Metoncholaimus demani	.	+	0·1	.
Oncholaimus dujardini	.	+	0·75	.
Oncholaimellus diodon	.	+	.	2·0
Oncholaimide juv.	.	.	+	.
Enchelidiidae:				
Eurystomatina filiformis	.	.	+	.
Symplocostoma longicollis	.	+	.	.
Catalaimus maxweberi	.	0·5	0·8	.
Cyatholaimidae:				
Cyatholaimus demani	1·4	0·5	1·5	6·0
Paracanthonchus coecus	.	4·0	0·3	.
P. kreisi	.	+	.	.
Choanolaimidae:				
Halichoanolaimus robustus	.	1·7	0·4	.
Desmodoridae:				
Desmodora serpentulus	.	4·8	1·0	1·0
D. scaldensis	.	1·0	+	.
Xenodesmodora porifera	.	+	.	.
Monoposthia costata	.	1·0	0·5	0·5
Microlaimidae:				
Crassolaimus bipapillatus	.	+	.	.
Comesomidae:				
Parasabatiera similis	.	.	.	0·5
Chromadoridae:				
Spilophorella paradoxa	.	1·8	2·8	0·5
Spilophora gracilicauda	.	0·3	0·7	.
Chromadorina parva	.	1·0	0·3	.
Euchromadora vulgaris	3·0	1·0	0·1	.
E. tridentata	.	0·3	+	3·5
Hypodontolaimus inaequalis	.	0·4	0·4	0·5
Prochromadorella neapolitana	.	.	.	1·5

TABLE X (*continued*)

Height (m.)	+4·50 to 3·25	+3·25 to 1·50	+1·50 to 0	0 to −3·00
Chromadoridae (*cont.*):				
Prochromadorella mediterranea	.	+	.	.
P. paramucrodonta	.	+	0·8	0·8
P. macro-ocellata	.	0·8	.	.
P. obtusidens	.	1·3	.	.
Chromadorella parapoecilosoma	.	2·6	1·0	.
C. microlaima	.	0·3	0·6	.
Neochromadora poecilosomoides	.	+	0·5	17·0
Chromadora nudicapitata	32·0	13·5	34·0	10·0
C. brevipapillata	.	.	0·2	29·5
C. macrolaima	.	0·6	0·1	.
Heterochromadora germanica	.	10·1	4·0	.
H. granulo-pigmentatus	.	0·8	0·2	.
H. cervix	.	0·3	.	.
Prochromadora longitubus	.	+	0·2	0·5
Axonolaimidae:				
Odontophora setosa	.	0·1	.	.
Araeolaimidae:				
Araeolaimoides paucisetosa	.	+	.	.
Camacolaimidae:				
Camacolaimus tardus	.	2·1	1·1	.
C. conicaudatus	.	+	+	.
Halaphanolaimidae:				
Dermatolaimus membranatus	.	0·6	.	.
Linhomoeidae:				
Linhomoeus elongatus	.	0·2	0·3	.
Paralinhomoeus lepturus	.	+	.	.
Metalinhomoeus typicus	.	+	.	.
Monhysteridae:				
Theristus acer	.	2·0	1·0	.
T. normandicus	.	+	.	.
T. setosus	.	.	.	4·0
Theristus sp.	.	+	.	.
Monhystera parva	.	+	0·2	.
M. luisae	1·1	.	.	.
M. refringens var. *britannica*	0·5	+	0·2	.
M. disjuncta	.	+	0·8	.
No. of samples	3	33	11	4
No. of specimens	262	4160	1327	156

olychaeta

In Table VIII is summarized the distribution of the polychaetes at Tinside he serpulids were not counted and are, therefore, omitted). It must be ressed that the absence of species in the lowest zone is not at all conusive since only relatively few specimens and only from one single biotope, iz. *Nitophyllum punctatum*, were studied.

By the same method as above the data from Colman's tables and Kitching's *istomus-Halichondria* association have been extracted (Table IX). Still ore striking than in Amphipoda is the abundance of species and individuals the *Laminaria* holdfasts which makes this habitat almost incomparable ith any other. Despite the differences between Tinside and Wembury Bay, in number of specimens studied and in the habitats sampled, two facts seem to be fairly well established. First, *Fabricia sabella* is the polychaete with by far the highest power of resistance to exposure, and it dominates the higher zones. Secondly, the level at about +2 m. is critical for nearly all other species, indicating that most polychaetes are fairly susceptible to exposure.

Nematoda

The distribution of the nematode fauna in the Tinside area has been dealt with in a previous paper (Wieser, 1951). It has been shown that nematodes are more dependent on the shape of the algae on which they live and on the silt content than any of the groups examined. The study of the vertical distribution is somewhat hampered by these facts since we are even less sure about the 'causae efficientes' of the presence or absence of a given species than in other animals.

All the available data, however, are summarized in Table X, giving the vertical distribution of the seventy species found at Tinside amongst 5945 specimens picked out from fifty-one samples. The uppermost and the lowermost zones were not so well studied as the two middle zones, and their data are thus less reliable. These deficiencies remembered, attention may be called to a few points.

(i) The most evenly distributed species are *Enoplus communis*, *Cyatholaimus demani* and *Chromadora nudicapitata*. (ii) In my opinion there are several species which can be called true infralittoral forms, viz. *Neochromadora poecilosomoides*, *Chromadora brevipapillata*, possibly *Theristus setosus* and *Prochromadorella neapolitana*. (iii) In two genera very closely related species seem to replace each other in the upper and lower part of the shore respectively, viz. *Euchromadora vulgaris* (high) and *E. tridentata* (low) and *Chromadora nudicapitata* (high) and *C. brevipapillata* (low).

CRITICAL ZONES

Colman (1933) and Evans (1947*a*, *b*) introduced and applied the term 'critical level' which accounts for the observation that 'certain levels (of the intertidal region) have been shown to be more critical than others in connexion with the distribution of intertidal plants and animals' (Evans, 1947*b*). Colman and Evans studied critical levels only in macro-organisms. In micro-organisms inhabiting seaweeds the problem becomes more complicated since the effect of the substratum, i.e. the seaweeds, on the vertical distribution of the fauna has to be taken into account. Where certain algae reach their upper limit most of the animals living among them will also find there the limit for their penetration into the intertidal zone. It is, however, not established whether this is due to the same change in the degree of tidal exposure which causes

e disappearance of the algae or to the fact that they are dependent on the
esence of the latter from a purely mechanical point of view. I therefore
ant to apply the term 'critical level', as far as the microfauna is concerned,
ly to those limits which do not correspond with the disappearance of algae.
can, however, be concluded from the data presented above that changes in
e algal fauna may reflect the influence of the substratum to a greater degree
an found in organisms attached to rocks. Differences in shape of the
aweeds cause the 'critical level' to oscillate somewhat in different habitats of
e same area so that one could rather speak of a critical 'zone'. Or, in other
ords, the range of variation of the upper and (or) lower limits of certain
imals seems to be greater in species inhabiting seaweeds than in those
ing as hemi-sessile or slowly moving animals on the surface of the
cks.

The five critical levels distinguished by Evans (1947*a*, p. 211 *et seq.*) are as
llows: (1) between M.L.W.S. and E.L.W.S., where the majority of intertidal
ecies achieve their lower limits; (2) between M.L.W.S. and M.L.W.N., which
arks the lower limits of certain other intertidal species; (3) just above
.L.W.N., where several sublittoral species reach their upper limits of penetra-
on into the intertidal zone; (4) just below M.H.W.N., marking the upper limit
one set of intertidal species; (5) between M.H.W.S. and E.H.W.S., where
further set of intertidal plants and animals achieve their upper limits.
ccording to what has been said above I must leave out levels nos. 4 and 5,
nce they would only concern species which disappear together with their
aweeds. Furthermore, levels (1) and (2) which overlap even in Evans's
gure (his p. 213) must be regarded as one as far as the microfauna is con-
rned. This I call 'Zone A', which is situated between M.L.W.S. and M.L.W.N.
the area under consideration. In this zone the true intertidal species which
ere found reach their lower limits.

I recognize an analogue of Evans's level no. 3, which I call 'zone B'
d which is situated between E.L.W.N. and M.T.L. This is slightly higher
pshore than Evans's level, for which the reason is believed to be the dense
fts of *Gelidium corneum* which allow several species to penetrate farther into
e intertidal region than they do in any other weeds examined. In this
zone B' a set of infralittoral animals reaches its upper limit.

A third 'zone C' deserves mention which either has no counterpart in
vans's survey or must be regarded as the lower part of his level 3 (though in
y samples well distinct from 'zone B'). It marks the upper limit of another
t of infralittoral species. It happens here to coincide with my 'zone A', but
should not be assumed that it necessarily does everywhere.

Summing up, the following 'critical zones' may be distinguished in the
inside area:

(A) Between M.L.W.S. and M.L.W.N., where several intertidal species reach
eir lower limits.

(B) Between E.L.W.N. and mean tide level, marking the upper limit of a set of infralittoral species.

(C) Between M.L.W.S. and M.L.W.N., where another set of infralittoral species achieves its upper limit.

The results are given in Fig. 1. It is only meant to show the one (upper or lower) limit of the species concerned that falls within the critical zone. This limit is indicated by broken lines marking somewhat deliberately the oscillations of the zone. The line 'bulk of *Fabricia sabella*' indicates the sudden decline in number of individuals of this species at about the +2·0 m. level (Table II), though single specimens might reach farther upshore.

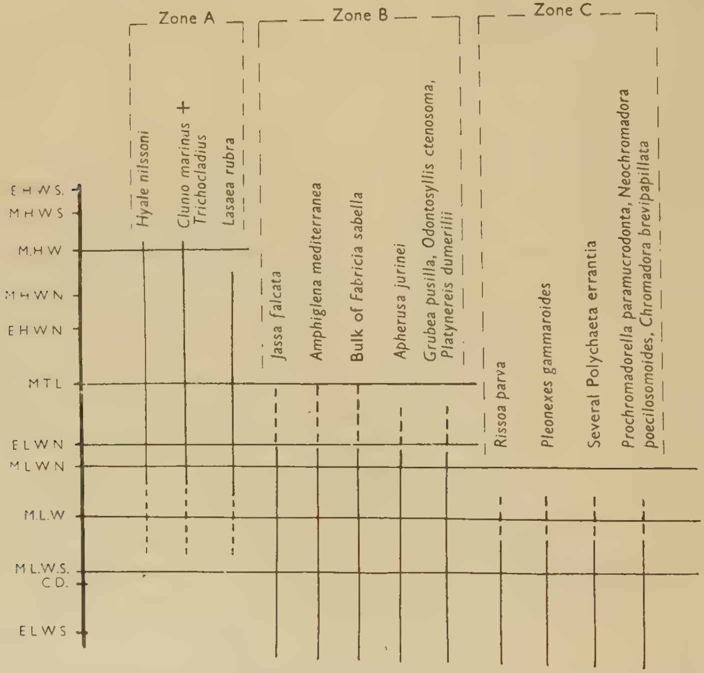


Fig. 1. Distribution of certain faunal types in relation to critical levels (see text for further explanation).

The following comparisons can be made with the data on the macrofauna published by Colman (1933), Evans (1947*b*) and Yonge (1949).

The species of zone A (*Hyale nilssoni*, *Clunio marinus*, *Trichocladius* cfr *vitripennis* and *Lasaea rubra*) are the counterpart of *Littorina littorea* *L. obtusata*, *Patella vulgata*, *Osilinus lineatus*, *Chthamalus stellatus*, *Ascophyllum nodosum*, and *Fucus vesiculosus*; the species of zone B (*Jassa falcata* *Apherusa jurinei*, *Amphiglena mediterranea*, *Grubea pusilla*, *Odontosylli* *nosoma, Platynereis dumerilii*) approximately coincide in their distribution th *Gibbula cineraria, Rhodymenia palmata, Gigartina stellata* and *Chondrus spus*.

The most interesting species, however, are those which achieve their upper nit in zone C (*Pleonexes gammaroides, Rissoa parva, Oridia armandi*, several lychaeta errantia, and nematodes like *Prochromadorella paramucrodonta, eochromadora poecilosomoides* and *Chromadora brevipapillata*), since they rrespond with the organisms of the 'infralittoral fringe' (Stephenson, A. & A., 1949), i.e. species which are typically infralittoral but nevertheless cupy a small fringe in the lower part of the intertidal region. To them long *Laminaria digitata, Himanthalia lorea, Pyura stolonifera* and most obably *Verruca stroemia* and *Calliostoma ziziphinum*, quoted by Colman 933). Furthermore, Yonge's 'average low tide level' (1949) coincides with e upper level of the infralittoral fringe and therefore, also, with my zone C upper limit of a set of infralittoral species) and zone A (as lower limit of some tertidal species). Briefly, this level between M.L.W.N. and M.L.W.S. appears be the most critical throughout the intertidal area: it is a true 'turning-int' of the highest ecological significance.

‘Dynamics’ of Vertical Distribution

the previous sections the distribution of the microfauna was dealt with om a purely 'static' point of view, i.e. the upper or lower limits of certain ecies or their level of maximum abundance was given by using the average lues of several samples disregarding the state of changes (rhythmical or oradic) which might occur in the environment. The result is an *average stribution* of the species in question which fully corresponds with the *actual stribution-area* in sessile organisms, to a very great extent also in hemi-ssiles, slowly moving and highly euryoecous species. But with active and elatively) stenoecous species care should be taken not to mix together mples which have been taken under quite different environmental conditions s, for example, height of tide), since by this method it is impossible to detect ovements which may counterbalance environmental changes if they become o unfavourable. In this case a more discriminating method should be applied d samples taken under different conditions should be kept separate. The term ctual distribution-area' therefore is meant to comprise all changes in the stribution of a given species correlated with fluctuations in the environment. aturally, certain restrictions have to be made, since over sufficiently long riods even sessile organisms extend or restrict their area of distribution for e special requirements of reproduction. Therefore only short-period fluctua-ons and their effect on some species will be discussed. Similar behaviour s been thoroughly investigated in movements of marine and limno-plankton connexion with changes of light intensity, etc.

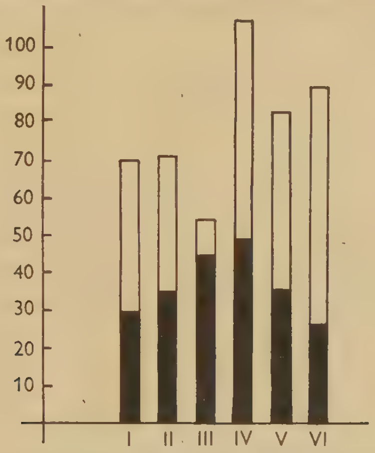
As is well known, tidal movements are of great importance in the littoral region. In areas with a big tidal range, in particular, extensive movements of the more mobile animals may occur with the rise and fall of the tide, but these are almost unknown in the microfauna. Watkin (1941) has shown the changes occurring in the arthropod fauna of a sandy intertidal area during high water. Two sorts of movements were found: the passive (and active?) upward transport by the rising tide of animals living in deeper water, and the (active) migration of true sand-dwelling species of the intertidal area into the waters above. With qualifications this can be compared with Remane's division into 'horizontal' and 'vertical migrants' (1940, p. 107).

It was possible to detect similar differences in the distribution of certain animals between low and high water on a rocky coast, such as at Tinside. This could be proved by collecting samples in several localities during low tide, and, by diving, during high tide. If the same differences of distribution occurred in all samples they could be regarded as significant. Naturally, not all the algae examined gave the same results. For example, in the small tufts of *Gelidium corneum* it was not possible to detect any differences in the composition of the fauna between high and low water. The most obvious differences were seen in the tall and shrub-like seaweeds *Gigartina stellata* and *Fucus serratus*. These weeds are most liable to desiccation when the tide is out and it is quite understandable that a set of mobile animals should leave them in this state and occupy them again on the succeeding flood. Those animals which cannot counteract the challenge of the environment in this active manner will mostly—as has been suggested in the general remarks at the beginning of this paper—find shelter in minute crevices, between the tiny roots and branches of epiphytes and epizoids.

I think it possible to distinguish two modes of migration within the fauna of seaweeds according to whether the animals are able to swim or merely able to crawl about. To illustrate the second condition I would refer to *Littorina obtusata*, the 'average' distribution of which is confined to the lower two-thirds of the intertidal area. The numbers of this species in twelve samples of *Gigartina stellata*, six of them taken during high tide, the other six during low tide, is shown in Fig. 2. Each column represents a different station within the area investigated. The whole column shows the number of specimens (per 20 g. dried weed) of the high-water sample, the black part that of the low-water sample at the same location. Thus the white part of each column represents the surplus of the flood samples over the ebb samples, and this appears to be quite significant.

These data strongly suggest that the snail carries out movements synchronous with the rise and fall of the tide. During high water it is more numerous on the fronds than during low water, when it seeks shelter within the denser and lower part of the seaweeds and in crevices of the rocky substratum nearby, escaping therefore detection if the seaweed is collected ese movements are explicable if we keep in mind that *Littorina obtusata* ds directly on algae and therefore the submerged and slowly floating fronds ovide a much better opportunity than the dry and shrunken shrub to ich tall algae like *Gigartina* are reduced during low water.

That it is the degree of humidity of the substratum and of the snail itself ich causes these migrations is supported by the results of Haseman (1911), o found the same oscillatory movements corresponding to those of the les in *Littorina littorea* at Woods Hole, Mass. This species feeds on small gae growing on rocks. It never crawls on dry surfaces and it has been shown various experiments that 'the primary directive force for rhythmical ovements is the surface film of water' (Haseman, 1911, p. 120). These rtical movements shown by *Littorina* make them comparable with Remane's ertical migrants', i.e. the species inhabiting an intertidal sandy area, which, hen the tide recedes, crawl downwards into the interstitial spaces of the nd-grains, thus remaining in a zone of optimal humidity.



g. 2. Numbers of *Littorina obtusata* in six low-water and six high-water samples. Each column represents a different station. On the ordinate the number of specimens per 20 g. of dried weed. (For further explanation see text.)

A similar mode of distribution, at least in appearance, is found in several pepods. But, since we know that copepods can swim ('bivagil' according to emane, 1940, p. 191), in addition to probable movements within the seaweed self, there are possibly more extensive migrations from one level to another, llowing the falling tide downshore and rising again with the flood. I am clined to regard this type of migration as prevailing in the copepods in view the abundance in plankton catches made by night of the species which e known to inhabit seaweeds (observation of Dr E. Dahl on the Swedish est coast, unpublished).

The present material was not adequate for studying several species parately, mainly because of the patchiness of their distribution (see also

Colman, 1940, p. 147). The distribution of the individual species is given in Table XI, while in Fig. 3 all the species are treated together. Again the high-water samples are represented by the whole columns and the low-water samples by the black part of them. (Owing to accidental loss of the tubes, a full examination of no. III and no. I from low water was not made.)

Table XI. Occurrence of Copepods in Twelve Samples of *Gigartina stellata*, Divided into High-water and Low-water Samples

(+ = present; + + = very common.)

	High water						Low water					
No. of sample ...	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Dactylopodia vulgaris	.	+ +	.	.	+	.	.	.	.	+	+ +	+ +
Idya minor	+ +	.	.	.	.	.	.	.	.	.	.	.
I. graciloides	.	.	.	+	.	.	.	.	.	.	.	.
Zaus spinatus	.	.	.	+	+	+	.	.	.	.	.	.
Saccodiscus littoralis	.	.	.	.	.	.	.	.	.	.	+	.
Rhynchothalestris rufocincta	.	.	.	.	.	.	.	+	.	.	.	+
Parathalestris clausi	.	.	.	.	+	+ +	.	+	.	.	+ +	.
P. harpacticoides	.	.	.	+	+	.	.	.	.	.	.	.
Laophonte similis	.	.	.	.	.	.	.	.	.	+	.	.
L. inopinata	.	.	.	.	.	.	.	.	.	.	.	+
Heterolaophonte sp.	.	.	.	.	.	.	.	+	.	.	.	.
Amphiascus sp.	.	.	.	.	.	.	.	.	.	.	.	+
Pseudonychocamptus koreni	.	.	.	.	.	.	.	.	.	.	+	+
Parastenhelia spinosa	.	.	.	.	.	.	.	+	.	.	.	.
Ameira longipes	.	.	.	.	.	.	.	+ +	.	.	.	.
Harpacticidae juv.	.	.	.	.	+	.	.	.	.	.	+	.
Oithona helgolandica	.	.	.	+	.	.	.	.	.	.	+	.
Acartia clausi	.	.	.	+	.	.	.	.	.	.	.	.
Total no. of specimens per 20 g.	31	53	32	65	50	31	12	14	30	14	25	18

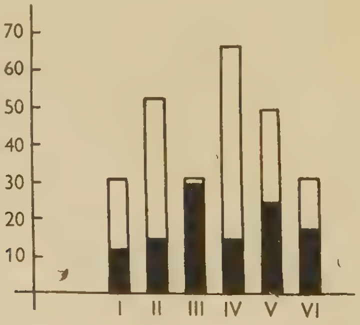
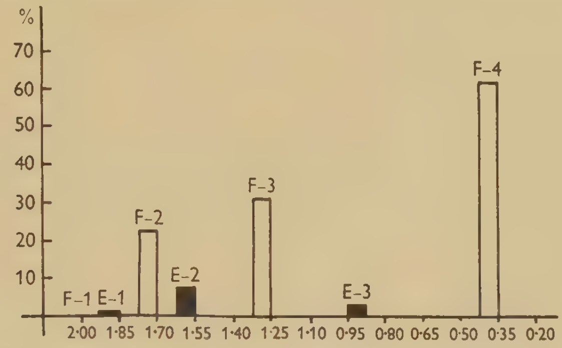


Fig. 3. Total Copepoda numbers at six stations, as in Fig. 2.

Since the copepods leave their seaweed cover with the receding tide and occupy it again on the flood we should reckon them (at least those species mentioned in Table XI) amongst Remane's 'horizontal migrants', though, with the exception of *Oithona helgolandica*, they do not come in from off-shore but only from lower levels of the algal zone. Nevertheless, a 'horizontal gradient' takes part in the movement and makes the analogy suggestive.

Finally, the vertical distribution of the amphipod *Stenothoë monoculoides* been studied in twenty-four samples, ten of which were taken during h water, from *Fucus serratus* and *Gigartina stellata*. The results are en in Fig. 4 and Table XII. Since the collecting was carried out more oroughly than in the former examples, I can give the exact height above C.D. the samples taken. Within a limited vertical range 2 to 5 samples were lected, the average value of which is represented by the columns in Fig. 4.



4. Distribution of *Stenothoë monoculoides*. Every column represents the average 'dominance value' of two to five samples within a limited vertical range. The mean vertical position of the samples above C.D. is shown by the position of the columns on the abscissa (metres above C.D.). Black columns represent low-water, white columns high-water samples.

LE XII. DISTRIBUTION OF *STENOTHOË MONOCULOIDES*. SPECIFICATION OF TWENTY-FOUR SAMPLES EXAMINED (FOR FURTHER EXPLANATION SEE TEXT)

	Low water			High water				
...	E-1	E-2	E-3	F-1	F-2	F-3	F-4	Total
ina samples	3	3	2	1	2	2	0	13
samples	2	2	2	1	1	1	2	11
ght above C.D. (m.)	2·0	1·9–1·4	1·3–0·7	2·0	1·8	1·6–1·0	0·7–0·2	2·0–0·2
nens in each	1, 0, 0, 0, 0	14, 2, 0, 0, 0	1, 1, 0, 0	0, 0	39, 2, 0	5, 72, 0	5, 20	162
inance value of	1	8	3	0	23	31	62	.

his time the dominance value of *Stenothoë monoculoides* in the whole mphipod population is given along the ordinate, since the algae were ergrown by epiphytes (*Elachistea fucicola*) and epizoids (*Membranipora embranacea*, *Dynamena pumila*) to very different degrees, making it impossible compare the numbers per weight-unit. (The numbers as well as 'dominance lues' are given in Table XII.) The data clearly show that the occurrence of e amphipod in the two algae considered, and between +2·0 and +0·2 m., much more abundant during high than during low water. Furthermore, ere might also be decrease in the density of the population upshore during gh water, pointing to the same susceptibility to desiccation which causes emigration from the intertidal area with the fall of the tide. For these reasons *Stenothoë monoculoides*, too, should be regarded as a 'horizontal migrant'.

SUMMARY

The vertical distribution of the microfauna inhabiting seaweeds in the Tinside area of Plymouth Sound has been discussed from various points of view. Samples were collected by diving below water-level (infralittoral and intertidal during high water) to ensure uniformity in the method of collecting.

A very strong influence of the substratum on the distribution of the fauna was observed. The extent to which different powers of locomotion of the animals concerned might help to counteract environmental changes is discussed.

The fauna of *Gelidium corneum* was studied in seventeen samples between +2·75 and +1·10 m. (above C.D.). Examples of the three possible relations to the factor of tidal exposure shown by littoral animals—(i) decline in number downshore, (ii) decline in number upshore, and (iii) more or less even distribution—are given.

The fauna of the two leaf-like algae *Porphyra laciniata* and *Nitophyllum punctatum* was studied in seven samples extending from +1·95 to −3·00 m. A most striking increase in population density was observed passing below low-water mark. Some species are indicated which are confined to the infralittoral or the littoral samples respectively.

The three tufted algae: *Ceramium* sp., *Cladophora rupestris* and *Lomentaria articulata* were studied between +3·25 and +0·8 m. in a total of twenty samples. Further support for the results gained from the *Gelidium* samples was obtained. The 'average' distribution of amphipods, polychaetes and nematodes in all samples studied is given. A comparison with published data on the fauna of Wembury Bay (Colman, Kitching, etc.) shows interesting agreement in some points.

It is pointed out that 'critical zones' for the distribution of the microfauna might occur in the area studied. Three zones are suggested, viz. zone A, between M.L.W.S. and M.L.W.N., where several intertidal species reach thei lower limits; zone B, between E.L.W.N. and M.T.L., marking the upper limi of a set of infralittoral species; zone C, between M.L.W.S. and M.L.W.N., wher another set of infralittoral species achieves its upper limit.

For each zone examples are given and comparisons drawn with the result obtained by Colman, Evans and Yonge.

The term 'dynamics of vertical distribution' is introduced, taking int account that various species might have a very different distribution in th intertidal area according to the state of the tide. The differences in th distribution between low and high water are suggested for *Littorina obtusat* several copepods and the amphipod *Stenothoë monoculoides*. The agreemen with the results obtained by Haseman, Watkin and Remane is discussed.

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APPENDIX. LIST OF ALL SAMPLES EXAMINED. SPECIFICATION, POSITION, NUMBERS OF AMPHIPODS, POLYCHAETES, NEMATODES AND TOTAL OF SPECIMENS.

Alga	Sample no.	Height relative to C.D. (m.)	Weight (g.)	Degree of silting (0–4)	Epi-growth (0–4)	Covered with water (+) or not (−)	Amphi-pods	Poly-chaetes	Nema-todes
Gelidium corneum	G-1	+2·75	1·3	3	0	−	6	49	1650
	G-2	2·75	2·2	0	0	−	54	·	472
	G-3	2·50	1·2	2	0	−	8	34	798
	G-4	2·50	1·0	2	0	−	18	26	600
	G-5	2·00	0·8	3	0	−	8	591	771
	G-6	2·00	1·4	3	0	−	2	445	920
	G-7	2·00	1·3	1	0	−	14	288	511
	G-8	1·90	1·2	3	0	−	4	587	765
	G-9	1·80	1·4	1	0	−	2	8	400
	G-10	1·75	1·3	3	0	−	·	723	856
	G-11	1·60	1·6	3	0	−	1	675	1200
	G-12	1·55	2·0	1	0	−	2	289	344
	G-13	1·50	0·6	1	0	−	·	227	224
	G-14	1·50	0·6	1	0	−	·	24	62
	G-15	1·50	1·6	3	0	−	1	458	779
	G-16	1·40	1·2	2	0	−	60	196	450
	G-17	1·10	0·8	4	0	−	42	35	668
Cladophora rupestris	T-1	+3·25	0·9	0	0	+	25	1	82
	T-2	3·25	0·9	0	0	+	22	2	76
	T-4	2·60	1·8	0	0	−	25	·	32
	T-6	2·50	2·0	0	0	−	40	1	107
	T-8	2·50	0·15	0	0	+	4	·	4
	T-9	2·50	1·2	0	0	+	14	·	5
	T-10	2·50	0·4	0	0	+	10	1	22
Ceramium sp.	T-3	+2·75	0·5	0	0	−	1	·	10
	T-5	2·50	1·0	0	0	−	34	·	120
	T-7	2·50	0·8	0	0	+	27	·	138
	T-11	2·25	2·0	0	0	−	·	·	187
	T-12	2·00	0·5	2	0	−	4	93	700
	T-13	2·00	0·5	2	0	−	3	84	548
	T-16	1·60	2·0	0	0	−	·	36	900
	T-17	1·60	1·7	0	0	−	·	·	66
	T-18	1·40	3·5	0	0	−	3	2	247
	T-19	1·40	2·6	0	0	−	·	·	302
	T-20	0·80	1·7	0	0	−	8	2	118
Lomentaria articulata	T-14	+1·90	2·0	1	0	−	6	32	62
	T-15	1·75	3·6	1	0	−	6	7	708
Porphyra laciniata	L-1	+1·95	35·0	0	0	−	1	·	13
	L-2	1·50	22·0	0	0	−	2	4	10
	L-3	1·50	16·0	0	0	−	3	1	3
Nitophyllum punctatum	L-4	−0·70	3·4	0	4	+	76	95	142
	L-5	−1·20	9·0	0	1	+	} 90	205 {	84
	L-6	−1·20	5·0	0	1	+			38
	L-7	−3·00	2·9	0	1	+	14	6	32
Gigartina stellata	S-1	+2·00	18	0	0	+	2	10	25
	S-2	2·00	35	0	1	+	23	61	95
	S-3	2·00	45	0	1	−	19	95	52
	S-4	2·00	40	0	?	−	1	19	7
	S-5	1·80	15	0	1	+	5	37	48
	S-6	1·80	22	0	2	+	66	20	36
	S-7	1·80	40	0	2	−	57	143	196
	S-8	1·80	55	0	3	−	16	154	273
	S-9	1·50	17	0	?	+	4	9	18
	S-10	1·50	3	0	2	−	1	17	5
	S-11	1·40	32	0	2	+	67	39	51
	S-12	1·40	40	0	4	−	30	52	119
	S-13	1·30	20	0	3	+	128	12	24
	S-14	1·25	60	0	4	−	85	213	588
	S-15	1·00	40	0	2	−	20	159	259
Fucus serratus	F-1	2·00	135	0	1	−	·	23	205
	F-2	2·00	24	0	1	−	2	3	18
	F-3	1·90	8	0	3	+	1	25	127
	F-4	1·90	25	0	0	+	1	2	52
	F-5	1·60	13	0	0	+	·	3	1
	F-6	1·50	20	0	0	−	·	1	16
	F-7	1·50	16	0	0	−	4	13	11
	F-8	1·40	65	0	1	−	1	19	56
	F-9	1·00	165	0	1	−	26	8	123
	F-10	1·00	21	0	0	+	·	·	1
	F-11	0·70	75	0	2	−	32	34	135
	F-12	0·70	28	0	3	+	27	16	200
	F-13	0·20	25	0	3	+	19	26	228

CONTRIBUTIONS TO OUR KNOWLEDGE OF THE SMALLER MARINE ALGAE

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(Plates I and II)

INTRODUCTION AND METHODS OF ISOLATION

ir lack of knowledge of nanoplankton organisms is due largely, no doubt,
the necessity for examining them alive and the comparative difficulty of
erving them, and also to their being, apparently, of no more than indirect
portance in the ecology of fishes, except when their occasional presence
great abundance has been accompanied by unfavourable hydrographical
anges. An adequate survey of life in the sea is bound to take them into
count. Since they are frequently abundant, at least in inshore waters, and
pagate rapidly, they probably form an important constituent of the food of
ult filter-feeding invertebrates. They are likely to be of still greater im-
rtance as the essential food of many planktonic larvae, particularly in the
ly stages. Oyster larvae (*Ostrea edulis* L.) can ingest nothing larger than
ut 10 μ and appear to rely for food on minute flagellates (Cole, 1936, 1939;
uce, Knight & Parke, 1940). The food of other marine larvae has not been
sely investigated.

Routine estimates of nanoplankton abundance have for some years formed
rt of investigations into the factors associated with good growth and survival
oyster larvae in the oyster-breeding tanks of the Ministry of Agriculture and
sheries at Conway and in oyster-producing estuaries of Cornwall and Essex.
he method used has been to concentrate a water sample quantitatively, by
ering through a Gradocol collodion membrane with the aid of an exhaust
mp, and then to count the organisms in subsamples of the concentrate, using
aemacytometer (Cole & Knight Jones, 1949). The work has been hampered
lack of information concerning the systematics of nanoplankton organisms.
e majority of those observed are of undescribed species and many belong
new genera. The affinities of some of the commonest minute flagellates are
scure. The present account is the initial result of efforts which are being
ade to fill this gap in our knowledge.

Most flagellates alter in shape and lose their flagella on treatment with many
atives and become virtually unrecognizable, though 1 % osmic acid may
ve satisfactory results; they must therefore be examined alive. Since they
e rapidly motile for long periods and can be studied adequately only under

a 2 mm. objective, the work has so far been confined to forms from whicl thick cultures could be obtained.

The culture medium used has been sea-water Erdschreiber (see Gross 1937). Serial dilution of sea-water samples into test-tubes of Erdschreibe proved to be the most useful method of isolating new cultures. This method outlined below, is based on that used in the bacteriological examination o water supplies (Ministry of Health, 1940).

For a nanoplankton of from 1 to 30 organisms per mm.^{3} (the density mos frequently encountered), a preliminary dilution of 1/100 was made; 1 ml. of th sea-water sample was added to 99 ml. of sterile sea water in a sterile stoppere bottle and shaken by inverting twenty-five times. Fifteen test-tubes, plugge with cotton-wool, sterilized and each containing 9 ml. of sterile Erdschreiber had previously been prepared and arranged in three batches of five. With sterile pipette 1 ml. of the preliminary dilution was added to each test-tube o the first batch. Then, with a fresh pipette, the contents were mixed by suckin up and down ten times and 1 ml. was transferred to a tube of the second batch This process was repeated for the remaining four tubes in each of the first an second batches, using the same pipette. Then with a fresh pipette and followin the same procedure, 1 ml. was transferred from each test-tube of the secon batch to a tube of the third batch.

The three batches of test-tubes then contained, respectively, dilutions o 1/1000, 1/10,000 and 1/100,000, i.e. approximately 10, 1 and 0·1 mm.^{3} of th original sea water. They were placed in windows facing north. Exposure t direct sunlight, even for short periods when the sun was low, or for a fe minutes only when the sun was high, often proved fatal to small flagellate A series of test-tubes hung in a south window, but not exposed to direc sunshine, did not develop such a variety of organisms as a similar series in north window. Apparently the wide temperature variations and the hig light intensity in south windows are unfavourable to many forms. Culture maintained at Nottingham grew well when kept in a cabinet at 12° C., unde constant illumination from two 20-W. warm white tubes.

It was found that thick cultures usually developed in some of the test-tube from the dilution series after 1–2 months in summer and 2–4 months in winte Those of the first batch generally contained mixtures of organisms, while thos of the third batch were often blank, but a few uni-algal cultures were usuall obtained from the second and third batches.

Subcultures were made every 2 or 3 months, into either test-tubes or 150 m flasks. Although cultures in the larger vessels usually appeared more thrivin and required less frequent attention, it was necessary, because of lack of spac to use test-tubes for the routine maintenance of cultures. It was found th many organisms could be maintained in mixed cultures, provided subculturin was carried out frequently. Some were subsequently isolated from these b making dilutions at favourable times. Some non-motile forms and larg gellates were isolated by picking out single cells or groups of cells with fine pettes under a high-power dissecting microscope, followed by washing in ccessive drops of sterile medium.

Platymonas apiculata n.sp. was positively phototactic when first observed, nd a few thousand specimens, introduced into a large volume of sterile sea ater, and left undisturbed, assembled on the side nearest to the light in a sible green cloud. Successive washings, taking advantage of this habit, oduced a culture which contained only one other flagellate.

Plates of solid medium (Erdschreiber with 2% agar) were prepared and oculated with diluted sea water. No growth of motile flagellates was btained, but *Nitzschia ovalis* Arnott, *N. longissima* Ralfs, *Synechocystis cillaris* n.sp., and an encysted strain of *Platymonas apiculata* were isolated by is method.

The quantitative dilution method described above is similar to that used bacteriologists for estimating the numbers of bacteria in water samples. count of the number of test-tubes developing cultures in each of the three tches indicates the probable number of organisms in the original sample. his can be found from statistical tables in the Ministry of Health's publication 940), which were recalculated and provided with their standard errors by waroop (1938). With nanoplankton organisms and present-day culture ethods it is unwarranted to assume that all the organisms introduced into the st-tubes survive and multiply, and this method has given estimated nano- ankton densities less than those obtained from haemacytometer counts of ncentrated samples.

In the taxonomic study of these organisms three things have greatly simpli- d their examination. The first was the discovery of a new method of staining gella. A drop of a suspension of the organism was placed on a slide and to is was added a drop of 1% osmic acid followed by a drop of a new methylene ue dye which was kindly supplied by Messrs Imperial Chemical Industries td. This dye is alkaline and soluble in sea water. It was found that the flagella most always stained a bright blue and the contents were often little altered.

The second discovery is due to Reynolds (1950), who has devised a lution which induces, in many algae, the formation of zoospores. This lution has been most useful in the identification of *Ulothrix* and other amentous forms.

The third discovery was that, if glass slips were inserted in the culture lutions in the test-tubes, many of the non-motile organisms readily grew on em and they could subsequently be withdrawn, examined and mounted, ore or less permanently, either stained or unstained. Inoculations of a new ulture could be made with a splinter of a glass slip chosen visually under a issecting microscope and the glass slip with adhering algae could be inserted n the spore-inducing solution with minimum disturbance.

The author's very best thanks are due to Mr Knight Jones, who was largely responsible for the introduction and the section on methods of isolation; also to him and Dr H. A. Cole for supplying and maintaining many of the cultures of the organisms here described; to Prof. Pringsheim for suggestions in the early stage of this work; to Dr M. Parke for cultures and suggestions; and especially to his friend Mr N. Y. Sandwith for great help in preparing the Latin diagnoses.

CHLOROPHYCEAE

CHLAMYDOMONADACEAE

Platymonas West

P. apiculata n.sp. (Pl. I, figs. 1–5).

Cellula compressa, a fronte visa late elliptica antice apice profunde biloba, flagellis 4 e sulco cylindrico exorientibus; chromatophoro campaniformi juventute laevis sed senectute granulis cum pyrenoide basali et multis amyli granulis dispersis praedito; stigmate singulo parte anteriore inserto, aurantiaco; cysto 1–2 cellulis cum membrana externa crassa et stratifera praedito. Cellulis 7·5–10·5 μ × 6·5–8·5 μ × 4·5–5 μ.

Cells elliptical or narrowly oval, somewhat compressed, apex two-lobed with a deep furrow from which the flagella arise; pyrenoid large, conspicuous, situated at base of cell; chromatophore campanulate, single, bi-lobed, smooth in young individuals but coarsely granular in cells from old cultures. Eye-spot situated in upper portion of cell, orange; cells contain also numerous starch grains and two to six deeply staining granules near the furrow similar to those recorded by Carter (1937) for *P. tetrathele* West; flagella four, arising from base of furrow, 1–1½ times as long as cell; cysts common in culture, one- or two-celled, surrounded by a thick, sometimes stratified, cell wall with an apical papilla.

In the shell-fish tanks at Conway, North Wales, in an aquarium at Lowestoft, and in the estuaries of the Helford River and the Crouch, apparently common.

This organism bears considerable resemblance to *P. gracilis* Kylin (1935) in size and position of eye-spot, but the apex is two-lobed and the presence of a papilla, usual in *Chlamydomonas*, has been recorded in this genus only for *P. cordiformis* (Carter) Korch. (Skuja, 1948) and it may well be present in other species. Carter (1937) is doubtful whether the lobing of the apex used by Kylin to divide the genus is a sound taxonomic character, but in all the strains of this new species which have been examined there is no doubt about the two-lobed apex and it would therefore appear correct to distinguish it from *Platymonas gracilis*.

The four strains of this organism which have been isolated vary somewhat in their behaviour in culture solutions. Every strain sooner or later becomes ncysted but, even under what are apparently identical conditions, the time that lapses before all motile forms do so varies with every culture. In young igorous cultures the chloroplast is almost smooth with very few starch grains ut, on ageing, starch grains become more numerous and large green granules ollect in considerable numbers in the cells.

The processes of division and encystment seem to be as follows. A cell ecomes quiescent and sheds its flagella, while at the same time the compresion becomes less obvious and the apical lobe shallow, so that, very shortly, the ell wall is entire and the cells scarcely compressed. Division takes place at an blique angle and as the cells grow they move round, so that in mature cells the dividing wall is often horizontal. The daughter cells acquire flagella while they are still within the parent cell, and may at times be seen moving about efore liberation. Liberation occurs by the splitting in half of the mother cell wall. When the cultures are old, single-celled cysts are the rule. These are not ompressed, have a thick stratified cell wall and are filled with starch granules nd other large spherical bodies which may possibly be oil.

CHLORELLACEAE

Chlorella Beijerinck

Unicellular green forms, of spherical or ovoid shape, are among the commonest of algae found developing in liquid cultures. Several such forms have een isolated from the River Crouch and elsewhere. All those now described ave been under observation for a long time and have been grown in several trengths of solution, including Reynolds's spore-inducing solution, and the nly form of reproduction which has been observed was by the production of non-motile bodies.

Subgenus *Euchlorella* Wille

C. *salina* n.sp. (Pl. I, figs. 11–14).

Cellula sphaeroidea, membrana externa tenui; chromatophoro laete viridi clare, phialiformi, parietali, cellulam fere implente; pyrenoide centrali, magna. Incrementum est per cellulae materis in 8 cellulas filias divisionem. Cellula 4–7 μ diametro.

Cells spherical; surrounded by an obvious but thin, smooth, cell wall; chromatophore saucer-shaped, almost filling cell, bright green, finely granular; pyrenoid central, large, surrounded by starch sheath.

Propagation by the successive division of the mother cell into eight daughter cells which free themselves by the bursting of the parent wall.

This organism developed in cultures from the Conway tanks. It bears a superficial likeness to C. *vulgaris* Beijerinck, but the cell wall is apparently thinner and the chromatophore rather different in shape. As it is a marine organism, it seems reasonable to distinguish it as a separate species.

C. stigmatophora n.sp. (Pl. I, figs. 30–34).

Cellula sphaeroidea vel aliquantum elongata, membrana externa tenui; chromatophoro laete viridi claro, phialiformi, cellulam fere implente sed depressione terminali praedito; pyrenoide manifesta, amyli capsula circumdata centrali nisi in cellulis elongatis, stigmate fusco, in cellulis parvis singulo, stigmatibus in cellulis majoribus 2 vel ultra plerumque terminalibus. Incrementum est per cellulae matris in 2 vel 4 cellulas filias divisionem. Cellula 4–6 μ diametro.

Cells spheroidal to somewhat elongate, 3–5 μ diameter, bright green, surrounded by smooth, thin cell wall; chromatophore saucer-shaped, filling most of cell but with depression at one end, rather granular; pyrenoid conspicuous, medium sized, central, though in the elongate cells rather to one end, surrounded by starch sheath; stigma single in young cells, two or more in the larger cells, usually at one end.

Propagation by division of mother cell into two, or more rarely four, daughter cells which are liberated by the bursting of the cell wall.

This was first isolated by Dr Parke (Bruce *et al.* 1940) from a sea-water sample collected at Port Erin, Isle of Man, and there referred to as *Chlorella* sp. Subsequently it has been found in the River Crouch, Essex.

In its size and method of division this plant is not unlike *C. spärckii* Alvik (1934), but it is clearly marked off from all other described species of *Chlorella* by the presence of at least one dark brown body which is interpreted here as a stigma.

Subgenus *Chloroideum* Nadson

C. ovalis n.sp. (Pl. I, figs. 15–22).

Cellula ovata vel ellipsoidea, membrana externa tenui; chromatophoro laete viridi, parietali, paulum lobato, cellulam ¾ implente. Pyrenoide nulla. Incrementem est per cellulae matris in 8 cellulas filias divisionem. Cellula 3–5 μ × 5–10 μ.

Cells ovoid to ellipsoidal, surrounded by a smooth hyaline cell wall; chromatophore parietal, bright green, smooth, slightly lobed, occupying about ¾ of the cell and, especially in young individuals, with a hyaline space at one end. Pyrenoid absent.

Propagation by successive division of the mother cell in individuals of 10 μ diameter into eight daughter cells which free themselves by the bursting of the cell wall.

First isolated from the River Crouch, Essex, and apparently common in that district.

This differs from *C. spärckii* Alvik in the division of the mother cell into eight and not two daughter cells. Another closely related species is *C. saccharophila* Kruger (1894), but from the somewhat inadequate description his organism seems to be 15–20 μ diameter with a granular chloroplast.

marina n.sp. (Pl. I, figs. 6–10).

Cellula ovata, membrana externa tenui, glabra; chromatophoro laete viridi, rietali, lobato, granuloso fere totam cellulam implente. Pyrenoide nulla sed rpore ut videtur olei in cellulis maturis. Incrementum est per cellulae atris in 8 vel 16 cellulas filias divisionem. Cellula 4–6 μ × 7–10 μ.

Cells ovoid, bright green, surrounded by smooth, hyaline cell wall; chroma- phore an irregular parietal plate occupying almost all the cell, markedly and ely granular. Pyrenoid not observed, but in the mature cells definite hyaline dies are present which appear to be oil.

Propagation by the successive division of individuals 10 μ in diameter into ght or sixteen daughter cells which are liberated by the bursting of the cell wall.

This organism appeared in the cultures of a flagellate originally isolated at rt Erin by Dr Parke.

This species is closely allied to the last from which it differs in its granular romatophore and spherical oil-like bodies.

Nannochloris Naumann

maculatus n.sp. (Pl. I, figs. 23–25).

Cellula sphaerica vel elongata, membrana externa tenui laevi; chromatophoro llide viridi phialiformi, granuloso fere totam cellulam implente. Pyrenoide lla visa; sed granulis amyli et oleorum. Incrementum est per cellulae atris in 2 cellulas filias divisionem. Cellula 3 μ diametro.

Mature cells spherical or slightly elongate, surrounded by smooth cell wall; romatophore pale green, cup-shaped, finely granular, occupying most of cell. o pyrenoid has been observed, but the cells contain both starch grains and her refractive bodies which are probably oil. Cells 3 μ diameter.

Propagation by division of the cell into two individuals of about 3 μ diameter.

First isolated from River Crouch, and common in saline ditches of that strict.

The genus *Nannochloris* was established by Naumann (1931) to include two ry minute *Chlorella*-like organisms in which the cells divided only into two, d there was no evidence of a surrounding mother-cell membrane. As far as n be ascertained in such small cells, the above species has this same charac- ristic, while in addition it has been possible to ascertain, in part, the internal ructure. Whilst it is about the same size and shape as Naumann's *Nannochloris ccoides*, its occurrence in sea water appears sufficient evidence that it is a fferent species.

atomus n.sp. (Pl. I, figs. 27–29).

Cellula minuta spheroidea membrana externa tenui; chromatophoro pallide ridi phialiformi, cellulam fere implente; pyrenoide non visa. Incrementum t per cellulae matris in 2 cellulas filias divisionem. Cellula 3 μ diametro.

Cells spheroidal, 2–3 μ diameter, pale green surrounded by smooth thin cell wall. Chromatophore saucer-shaped, filling almost the whole cell, finely granular. Pyrenoid not observed.

Propagation by division of cells 3 μ diameter into two daughter cells.

First isolated in dilution cultures from River Crouch and apparently widely distributed in that district.

This is another very minute member of the Chlorellaceae which has the same general characters as the last except that it is even smaller and the numerous starch grains and similar bodies are apparently absent.

Stichococcus Naeg.

S. cylindricus n.sp. (Pl. I, fig. 26).

Cellula cylindrica membrana externa tenui, laevi; chromatophoro laete viridi, parietali, laevi, cellulam totam implente. Pyrenoide nulla; nucleo minuto. Incrementum est per cellulae matris in 2 cellulas filias divisionem; filamento brevissimo, facile fracto. Cellula 2 μ × 3–4 μ.

Cells cylindrical with rounded truncate ends, cell wall thin, hyaline; chromatophore parietal, bright green, smooth, completely filling cell; pyrenoid absent; nucleus small, difficult to detect.

Propagation by the transverse division of the mother cell into two equal halves; filament very short, easily fragmenting. Cells 2 μ × 3–4·5 μ.

Isolated from River Crouch, Essex.

This organism in many respects occupies a position intermediate between a species of *Nannochloris* such as *N. bacillaris* Naum. and *Stichococcus bacillaris* Naeg. which shows a similar slight tendency to form filaments. A very thin mother-cell wall can occasionally be distinguished between the cells, though it appears to be short-lived. Absence of such a mother-cell wall is one of the chief characters of the former genus. Species of *Stichococcus* are usually terrestrial and this form, being both aquatic and marine, is clearly a well-marked new species.

CHRYSOPHYCEAE

CHROMULINACEAE

Chromulina Cienkowsky

C. pusilla n.sp. (Pl. II, fig. 42).

Cellula minuta, sphaeroidea dorsiventraliter paulum depressa, chromatophoro singulo, parietali, phialiformi, fulvo; flagello singulo, 2–3 μ longo ut videtur secus marginem superiorem lateris applanati inserto; corpore fusco centrali. Cystis sphaeroideis membrana externa laevi. Cellula 1–1·5 μ diametro.

Cells rounded, with definite dorsi-ventral flattening, 1–1·5 μ diameter periplast firm. Chromatophore single, parietal, saucer-shaped, filling most o ell. Flagellum single, 2–3 μ long, apparently inserted along upper edge of attened side; chromatophore brownish green, single, apparently a parietal late, filling about two-thirds of cell. The only other cell structure which can e made out in this small organism is a central dark granule the nature of which is difficult to decide. Cysts endogenous, rounded, wall smooth, unsculptured. Propagation by vertical division of the cells.

This, or some organism closely related to it, is apparently widely distributed, s it has been obtained repeatedly in cultures from Conway, the Helford River, Cornwall, and the River Crouch, Essex. The greatest density recorded by the erial dilution method previously described was 3500 per ml. in October 1946 rom Cornwall. In addition, minute flagellates probably of this species are egularly observed in plankton counts from the Conway tanks and the Essex stuaries.

The assignment of this organism to its true position is a matter of some ifficulty as, in so small a species, the cell contents are very hard to define. The general brownish colour of the suspensions in mass and the greenish rown colour of the individuals, together with the simple organization of the ells, suggest a close relationship to *Chromulina*, an alliance also confirmed by he apparent formation of cysts. Against placing the organism in this genus is he unilateral compression of the cells and the apparently lateral insertion of he flagellum. Though these are important characteristics, the fact that *Chromulina* includes species of varied shape seems to justify the inclusion of his species within the genus.

C. pusilla differs from *C. pleiades* Parke (1949) in having one chromatophore nly, as well as in the absence of vibrating bodies, and it differs from this and *C. parvula* Conrad (1930) by its flattened side, and from all other described pecies in its very small size.

OCHROMONADACEAE

Pavlova n.gen.

Cellula solitaria, natante, nuda periplasto differentia carente praedita forma mutabili; 2 flagellis, dissimilibus, plusminusve lateraliter insertis.

Cells solitary, motile, naked with undifferentiated periplast; strongly metabolic. Flagella 2, unequal, inserted more or less laterally.

P. gyrans n.sp. (Pl. II, figs. 35–38).

Cellula mutabili, elongata, aut ovata, aut amoeboidea, compressa, posteriore acuta; chromatophoris 2, magnis, fulvis, lateralibus, cum multis granulis dispersis; flagellis 2 dissimilibus, plusminusve lateraliter insertis; altero longiore apicem versus, altero breviore extrorsus provecto; parte apicali anteriore vacuolo contractili et stigmate singulo rubro praedita; parte basali posteriore granulis leucosini 1–2 praedita. Cellula 3–6 μ × 4–10 μ.

Cells strongly metabolic, either elongated, ovoid or amoeboid, usually compressed, base acute; chromatophores two, large, lateral, not extending to the base, dull yellow, granular; flagella two, unequal and inserted at some distance below the apex, the larger directed towards the apex and the shorter outwards; a conspicuous red stigma and a small contracting vacuole are present in the apical region; at the posterior end one to three leucosin bodies are present.

Propagation is by longitudinal division in the non-motile stage.

Isolated from the Helford River, Cornwall, in 1947.

This flagellate is most closely allied to the metabolic forms of *Ochromonas*, but differs in the sublateral insertion of the flagella, a character deemed of sufficient importance to separate it from this genus.

The metabolism of the individuals is so marked and the shape so varied that the form of the cells is anything from cylindrical compressed to almost spherical and amoeboid. Only the most frequently observed shapes are illustrated (Pl. II, Figs. 35–38).

Of the two flagella the one directed forward has a somewhat slow undulatory motion and can be seen without difficulty. It is about twice as long as the cell. The smaller flagellum is directed outwards and has a rapid up and down movement; in length it about equals the width of the cell and is very much more difficult to detect than the longer flagellum. The movements of the organism are very varied. Sometimes it twists and turns on its own axis, or goes forward with a vibratory motion, while at other times it moves rapidly in one direction. Or it may execute a combination of any of these movements.

A palmelloid state has been occasionally observed in cultures. The outline of the cells is then very irregular, but stigma and leucosin are very conspicuous.

The cells are very sensitive to all fixatives, in which they either become spherical or burst. In excess light on the microscope slide the cells behave as follows. Motion ceases and the elongate cells become oval or spherical, the long flagellum floats away and part of the hyaline contents of the cell is extruded through the periplast to form elongate or club-shaped 'pseudopodia'. Some individuals do not immediately lose their power of motion but continue to move with pseudopodia protruding. Apparently this form of the organism may best be considered a pathological condition.

CRYPTOPHYCEAE

CRYPTOMONADACEAE

Hillea Schiller

H. marina n.sp. (Pl. II, figs. 39–41)

Cellula spherica vel ovata, lateraliter depressa; depressione lata haud profunda a margine apicali lateris applanati deorsum per quartam cellulae partem te decurrente; 2 flagellis disparibus ex apice depressionis exorientibus; omatophoro singulo, disciformi; parietali, fulvo, lobato granulis praedito; cleo posteriore manifesto; pyrenoide centrali. Cellula 2 μ × 2·5 μ.

Cells spherical to ovoid with distinct lateral flattening, rounded or tapering the base; a broad shallow depression runs vertically from the apical margin the flattened side and dies out one-quarter of the length of the cell; flagella o subequal, arising from apex of depression; chromatophore single, parietal, ed, granular, dull yellow; nucleus distinct, situated towards the base; renoid central. Fission not observed.

Isolated from sea water in the shell-fish tanks at Conway, North Wales.

This organism is very small and its complete structure is not easy to resolve. s salient features are the subequal flagella, the pyrenoid and the shallow pression. The first two are characters of the Cryptomonads. The depression very shallow and rudimentary and no trichocysts can be seen on its margin. can hardly therefore be considered a furrow. Schiller (1925) describes a ther larger organism from the Adriatic, *Hillea fusiformis*, which apparently s a depression on one side and a conspicuous nucleus. The species now escribed appears to have characters sufficiently similar to justify its inclusion the same genus. In shape and size, however, it is quite unlike *H. fusiformis* hiller.

Cryptochrysis Pascher

. *fulva* n.sp. (Pl. II, figs. 43–45).

Cellula nuda, natante, ovata dorsiventraliter depressa, asymmetrica; sulco ne notato a margine anteriore paulo infra apicem perduas tertias partes ginae lateralis decurrente; ordinibus trichocystorum duobus in margine lci; deest gula; 2 flagellis disparibus in sulco insertis; chromatophoro ngulo, magno, plicato, lobato, fulvo; pyrenoide plerumque singula vel in llulis majoribus 2, centrali, amyli capsula circumdata; nucleo parvo centrali l posterius inserto. Cellula 3–4 μ × 5–7 μ.

Cells naked, actively motile, ovoid in dorsal view, slightly flattened on one de though sometimes tapering to the base or with a somewhat truncate apex. well-marked furrow runs obliquely from a short distance below the apex nd dies out two-thirds down the lateral surface; two lines of trichocysts on ge of furrow; gullet not observed; flagella two, subequal, 10–12 μ long, ising from lower edge of furrow; chromatophore single, large, folded, lobed, eenish brown; pyrenoid usually single, or two in larger cells, central or one either end, surrounded by a starch sheath; nucleus small, situated in middle lower part; contractile vacuole apical, small, single. Multiplication by ongitudinal division in motile stage and possibly also in the palmelloid state.

Isolated from the sea water in the shell-fish tanks at Conway, North Wales, 946.

As noted by Pringsheim (1944) for other species, this and the two following organisms form palmelloid states in culture, in which the cells are irregularly massed, and the contents are of a richer brown than in the motile cells, containing what appear to be oil granules as well as the pyrenoid. This condition appears to correspond very closely with the palmelloid state of the alga described by Reinisch (1911) as *Phaeocapsa marina* = *Phaeoplax marinus* Pascher (1911), and there seems to be little doubt that the two organisms are very closely related; in fact, a somewhat rounder apex and a sublateral insertion of the flagella seem to be the chief distinguishing points of my species. While the conditions which bring about this palmelloid state have not been ascertained the history of one culture may be relevant. Isolated in April 1948 it continued motile and gave satisfactory subcultures until 9 September when a brown deposit was seen in the original culture. This was found to be the palmelloid state together with a number of motile cells. A subculture made from this produced only the palmelloid state from 2 October onwards and was discarded in the following March. In the meantime the original culture remained in the palmelloid state with no vestige of a motile cell from October 1948 to May 1949, when motile cells were again seen, being released singly from the palmelloid cells. Meantime two other more recent cultures which had also been palmelloid had become full of motile cells though some 3 months younger. All these cultures, and any subcultures made from them, remained motile throughout the summer months.

It thus seems that the organism can multiply both in the motile and palmelloid stages, and the question arises whether the genus *Phaeoplax* should be retained. The ease with which other algae are known to form strange palmelloid states in culture, the inconstancy in nature of the formation of such structures, as for example the mucilage tubes in certain diatoms, and the frequency and apparent longevity of the motile state, are against the retention of the genus; the fact that multiplication is possible in the palmelloid state is the chief point in its favour. This and the two following similar organisms apparently do not possess a gullet and are thus included in the genus *Cryptochrysis*, and it seems logical that the species described by Reinisch should be called *C. marina*.

The chief distinguishing characters of this species are the single chromatophore, the general shape, and the sublateral insertion of the flagellum.

C. lateralis n.sp. (Pl. II, figs. 48–50).

Cellula nuda, natante, ovata vel obconica lateraliter depressa, asymmetrica, postice acuta; sulco bene notato de margine dorsali triente infra apicem fere usque ad basin paginae ventralis fere recte decurrente; ordinibus trichocystorum duobus in margine sulci; deest gula; 2 flagellis disparibus in sulco insertis; chromatophoris 2, magnis, lobatis, fulvis; pyrenoide singula, centrali, amyli capsula circumdata; cellulis senioribus granulis amyli dispersis praeditis; parte cellulae inferioris hyalina, granulis vel ultra refractivis proedita; nucleo postice inserto; cellula 3–4 μ × 5–7 μ.

Cells ovoid to obconical in dorsal view, slightly compressed with lateral attening, tapering to the base; a well-marked furrow runs almost vertically rom the ventral margin, one-third from the apex and dies out almost at the base on the lateral surface; two lines of trichocysts on edge of furrow; gullet not observed; flagella two, subequal, arising from the lower edge of furrow, –10 μ long; chromatophores two, large, lobed, filling most of cell, greenish brown; pyrenoid usually single, medium, obvious, surrounded by starch sheath, more or less centrally placed; older cells with scattered starch grains in addition; contractile vacuoles not observed. Lower portion of cell hyaline containing one or more oscillating refractive granules. Multiplication by longitudinal division, in motile state.

Isolated in August 1946 from the shell-fish tanks at Conway.

This species also forms palmelloid states in culture similar to the previous species. Though the insertion of the flagella is almost as low as in the Nephroselmidaceae, this organism is more closely related to the previous species than o the former group and can be easily distinguished by the shape, the two chromatophores, and the almost vertical furrow.

C. *virescens* n.sp. (Pl. II, figs. 46, 47).

Cellula nuda, natante, ovata, dorsiventraliter depressa; sulco lato bene notato margine dorsali circiter tertia parte infra apicem exoriente atque fere usque ad mediam paginam lateralem oblique decurrente; ordinibus 2 trichocystorum haud bene notatorum in sulco; deest gula; 2 flagellis subaequalibus margine sulci insertis, 6–10 μ longis; chromatophoro singulo totam cellulam occupante, viridi, glabro; pyrenoide singula, magna, basali; granulis in cellulis senioribus obviis; cellula 3–4 $\mu \times$ 5–7 μ.

Cells naked, roundly ovoid, somewhat flattened on ventral side, asymmetrical; a wide well-marked furrow runs obliquely from the lateral margin, about one-third from the apex and dies out about the centre of the lateral surface; two lines of faint trichocysts on the edge of furrow; gullet not observed; flagella two, subequal, arising from the edge of furrow, 6–12 μ long; chromatophore single filling the whole cell, green, smooth; pyrenoid single, large, basal; globules possibly oil are present in older individuals. Multiplication by longitudinal division, apparently in the motile state.

Isolated on one occasion from the River Crouch, Essex.

This species can be recognized at once by its colour which, in spite of the wide variation, is unusual in the Cryptophyceae. The furrow is very wide and shallow and this, in apical view, gives the cell a reniform appearance. The dorsiventral compression is also unusual. Nevertheless, its structure with furrow, subequal flagella, large pyrenoid and general appearance is clearly that of a member of the genus *Cryptochrysis*.

Cryptomonas Ehrenberg

C. acuta n.sp. (Pl. II, figs. 51–53).

Cellula nuda, natante, elliptica, dorsiventraliter depressa, basi acuminata vel acuta, apice acuta vel rotundata; sulco bene notato margine ventrali fere quarta parte infra apicem exoriente atque fere usque ad basin fere recte decurrente; ordinibus trichocystorum in sulco valde distinctis; gula sulci tret-quartras partes longitudinis occupante; 2 flagellis subaequalibus in stulco insertis 12–15 μ longis; chromatophoris 2 magnis leviter lobatis, totam cellulam basi excepta occupantibus, granulis paucis exceptis laevibus, fuscis; pyrenoide magna centrali vel parte anteriore inserta, amyli capsula circumdata; nucleo parvo, parte anteriore inserto, granulis basi cellulae oscillantibus. Cellula 12–15 μ × 4–6 μ × 5–7 μ.

Cells actively motile and slightly metabolic, elliptical with some dorsiventral flattening, base acuminate or acute, apex acute or rounded; a well-marked furrow runs almost vertically from the ventral side a quarter of the length from the apex and dies out almost at the base; trichocysts lining the furrow very distinct; gullet in furrow and three-quarters of its length; flagella two, subequal, arising from the upper edge of the furrow, 12–15 μ long; chromatophores two, large, slightly lobed, occupying all except basal portion of the cell, smooth except for a few granules, brown; pyrenoid large, central or in the upper portion, surrounded by starch sheath; nucleus small, in upper part of cell; basal part of cell hyaline and filled with oscillating granules. Multiplication by longitudinal division, apparently only in non-motile state.

Isolated both from the River Crouch and the Conway tanks, but apparently not common.

The gullet in this species is not very marked and it may prove to be intermediate in this respect between the genera *Cryptochrysis* and *Cryptomonas* and justify the suggestion of Pringsheim (1944) that it is unreasonable to keep them separate. Oscillating granules similar to the well-known structures in desmids have now been recorded in Chrysophyceae by Carter (1937) and Parke (1949), and this species is an interesting example in the Cryptophyceae. Similar structures, though not actively oscillating, were also apparently present in *Cryptochrysis lateralis* (see p. 187).

The distinguishing features of this form are the tapered ends, a shape which is rare in this genus and shown only by *C. gracilis* Skuja (1948), the oscillating bodies and the almost vertical furrow. The sublateral origin of the flagella is also unusual. The presence of a pyrenoid is the most obvious distinction between this and *C. gracilis* Skuja.

This plant is very sensitive to light and certain chemicals. It disintegrates within 5 min. under the microscope, the pathological symptoms being the extrusion of the protoplast in the form of pseudopodia. It does not change its shape on treatment with iodine but immediately assumes a spherical form on the addition of New Methylene Blue.

MYXOPHYCEAE

CHROOCOCCALES

Several very minute blue-green organisms with the cells apparently not enveloped in a mucilaginous mass appear from time to time in cultures. One such organism has been identified with *Synechococcus elongatus* Naeg., while others are suggestive of *Pediochloris* Geitler and *Tetrachloris* Pascher, members of the so-called Chlorobacteriaceae. They are often very minute and it is possible that, before their nature can be fully elucidated, methods akin to those used in the taxonomy of bacteria will have to be adopted. The following, however, appears to be a well-marked undescribed species.

Synechococcus Naegeli

S. bacillaris n.sp. (Pl. II, fig. 54)

Cellula parvula, singula vel e filamentis brevissimis constante, fere spherica, ovata vel cylindrata; deest vagina mucilaginosa; periplasto firmo, mucoso, granulis tenuibus coerules viridibus cellulam implentibus. Incrementum est per cellulam in 2 cellulas transverse divisam. Cellula 1·5 μ × 1·7–4·5 μ.

Cells single or in very short filaments, not enveloped in a sheath of mucilage, almost spherical, ovoid or cylindrical, longest immediately before division; cell wall firm, apparently a thin mucous coat; contents finely granular, uniform, blue-green. Propagation by transverse division of the cells.

First noticed in June 1947 in a tube inoculated with a greenish brown colony from a culture from the Conway tanks growing on a solid medium.

With its nearly spherical cells this species occupies a position between the genera *Synechocystis* Sauvageau, in which all the cells are spherical, and *Synechococcus* Naegeli, in which the shape varies between ovoid and cylindrical. In size it is nearest to *S. elongatus* Naeg. (also isolated at Conway) but the cells are only shortly cylindrical and never elongated.

SUMMARY

Sixteen species of marine algae have been isolated by dilution culture from various coastal waters and are described. They include seven flagellates.

Four are species of *Chlorella* (*C. stigmatophora* n.sp., *C. salina* n.sp., *C. ovalis* n.sp., *C. marina* n.sp.): two are species of the closely allied genus *Nannochloris* (*N. maculata* n.sp. and *N. atomus* n.sp.) and one is a species of *Stichococcus* (*S. cylindricus* n.sp.). A new species of the Chlamydomonadaceae (*Platymonas apiculata* n.sp.) is also described. Two others are members of the Chrysophyceae; a very minute form 1–2 μ diameter, namely *Cromulina pusilla* n.sp., and an Ochromonad with lateral flagella (*Pavlova gyrans* n.gen. et sp.). The Cryptophyceae are represented by three species of *Cryptochrysis* (*C. fulvus*, n.sp., *C. lateralis* n.sp. and *C. virescens* n.sp.), a species of *Hillea* (*H. marina* n.sp.) and a species of *Cryptomonas* (*C. acuta* n.sp.). A minute blue-green algae, *Synechococcus bacillaris* n.sp., is also described.

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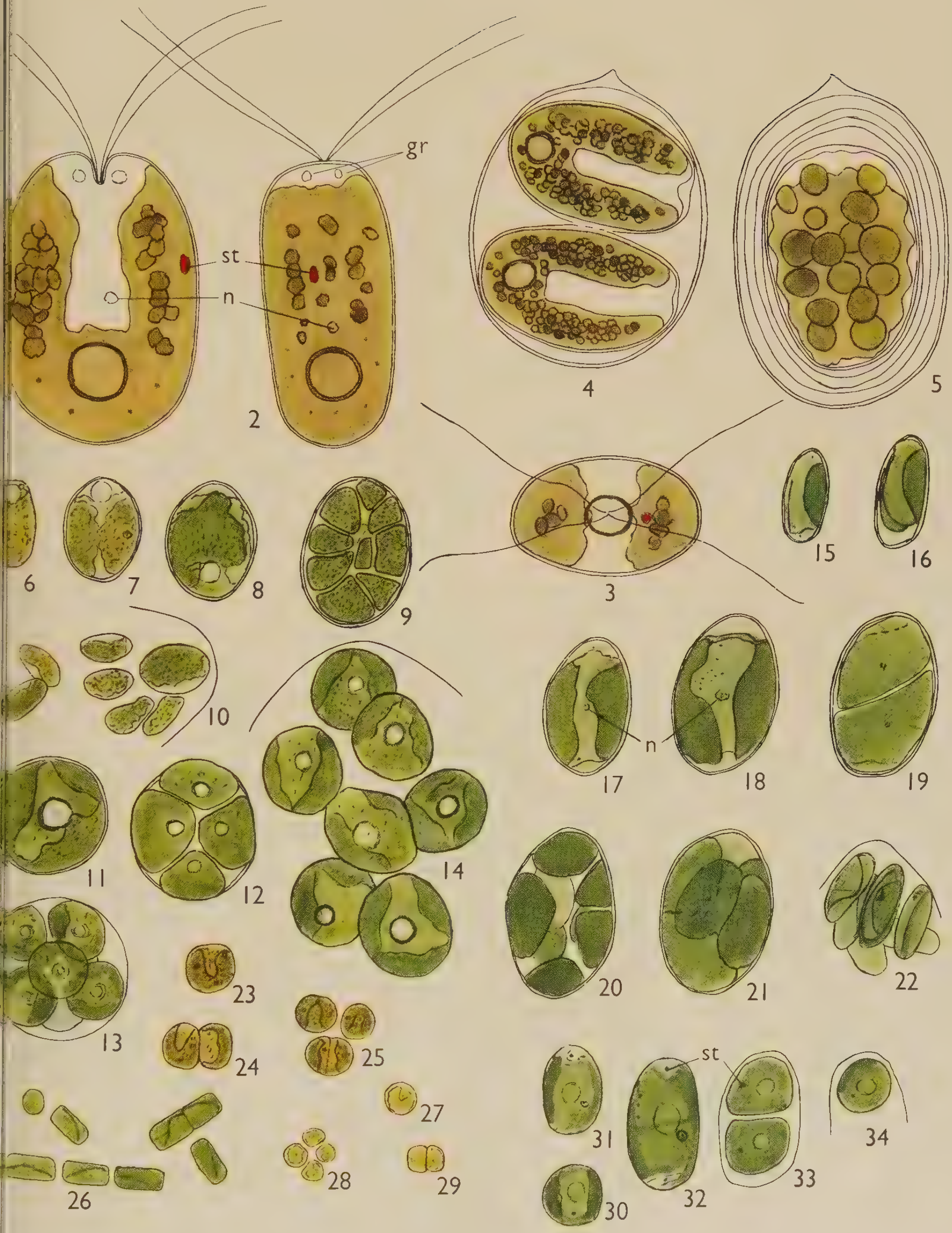
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EXPLANATION OF PLATES I AND II

PLATE I

(*gr.* = granules; *l.* = leucosin; *n.* = nucleus; *p.* = pyrenoid; *st.* = stigma; *cy.* = cyst.)

gs. 1–5. *Platymonas apiculata* n.sp. Fig. 1, front view; fig. 2, side view; fig. 3, anterior view; fig. 4, cyst; fig. 5, older single-celled cyst with stratified wall. All × 4500.

gs. 6–10. *Chlorella marina* n.sp. Figs. 6–8, cells of varying size; figs. 9, 10, stages in division. All × 2250.

igs. 11–14. *Chlorella salina* n.sp. Figs. 12–14, stages in division. All × 2250.

igs. 15–22. *Chlorella ovalis* n.sp. Figs. 15–18, cells of different size; figs. 19–22, stages in division. All × 2250

igs. 23–25. *Nannochloris maculatus* n.sp. × 2250.

ig. 26. *Stichococcus cylindricus* n.sp. × 2250.

igs. 27–29. *Nannochloris atomus* n.sp. All × 2250.

igs. 30–34. *Chlorella stigmatophora* n.sp. Figs. 32–34, stages in division. All × 2250.

PLATE II

igs. 35–38. *Pavlova gyrans* n.gen. et sp. Figs. 35, 37, two individuals in lateral view; fig. 36, another individual, ventral view; fig. 38, anterior view. All × 4500.

igs. 39–41. *Hillea marina* n.sp. Fig. 39 dorsal view; figs. 40, 41, lateral view. All × 4500.

ig. 42. *Cromulina pusilla* n.sp. × 4500.

igs. 43–45. *Cryptochrysis fulva* n.sp. Figs. 43, 45, lateral views; fig. 44, ventral view. All × 4500.

igs. 46, 47. *Cryptochrysis virescens* n.sp. Fig. 46, lateral view; fig. 47, anterior view. Both × 4500.

igs. 48–50. *Cryptochrysis lateralis* n.sp. Figs. 48, 50, lateral views; fig. 49, ventral view. All × 4500.

gs. 51–53. *Cryptomonas acuta* n.sp. Fig. 51, lateral view; fig. 52, another individual, ventral view; fig. 53, the same, lateral view. All × 4500.

ig. 54. *Synechococcus bacillaris* n.sp. × 4500.

THE AQUARIUM AND SEA-WATER CIRCULATION SYSTEM AT THE PLYMOUTH LABORATORY

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(Plate I and Text-figs. 1–4)

INTRODUCTION

The aquarium or tank room, to which visitors are admitted on payment of a small charge, was not designed primarily for public display, but was intended principally to facilitate scientific observations on the habits and life histories of marine animals. This original purpose it has never lost, but of recent years it has increasingly catered also for the steadily growing number of people interested in natural history, and for numerous classes of school-children brought by their teachers. Since the aquarium was re-opened in November 1946 attendances have shown a big increase over comparable pre-war figures.

The aquarium is over sixty years old. The fact that during the whole of that time the same tanks, the same reservoirs and much of the same piping have continued to serve without major trouble is a tribute to its designer and builders. The circulating system still in use was invented by W. Lloyd and was widely adopted for the public aquaria which were a popular feature of many towns towards the end of the last century. The system was also adopted at Naples. That is not to say, however, that the design and construction at Plymouth are ideal. Long experience, greater knowledge and changing function all emphasize the desirability of alterations for the benefit of the inhabitants of the tanks, for greater efficiency in working and for better viewing conditions for the observers, be they naturalists or general public.

Some improvements have been made since the war, during the repair and re-establishment necessitated by the damage suffered during air-attacks in 1941. These chiefly concern the erection of new rockwork in several of the arge tanks, and the provision of boards along the top edges of the latter to shield the viewer's eyes from window-glare. Electric lighting for dull days has also been provided.

That the reputation of the Association's aquarium stands high is shown by he numerous inquiries concerning its size, construction and maintenance eceived during the post-war years. Promoters and architects for aquarium chemes at home and abroad have written, or called for information and advice. Some have even brought plans for criticism. It is probable that not a few large public aquaria projected or building have been influenced in design by ideas and suggestions based on experiences at Plymouth. This world-wide interest in aquarium construction and management, and the rising numbers of visitors to our own aquarium, implies a need for a fairly detailed published account of the Plymouth aquarium as it exists to-day. Many details are equally applicable to marine aquaria anywhere, and should therefore be of interest to planners of all large installations.

THE AQUARIUM OR TANK ROOM

The last fairly detailed account published is that by Allen & Harvey (1928). The dimensions they gave were from an old plan and differ a little from those given here, based on new measurements. Widths and lengths of tanks are given to the nearest ¼ ft.; these measurements are all internal. The reader will find it helpful to refer frequently to the plan and section shown in Text-figs. 1 and 2.

The tank room or aquarium (Pl. I) is situated on the ground floor of the south building. It is a single large room measuring internally 70 ft. × 30 ft. (Allen & Harvey give the width as 34½ ft., but this includes the thickness of the walls), with a minimum ceiling height of 11¼ ft. The ceiling consists of seven shallow vaults. The room is entered and left by a wide doorway at the west end (seen in the photograph). A private doorway at the east end is normally kept locked.

South-Side Tanks

The whole of the south side is occupied by nine tanks built close against the wall. All are a little over 4 ft. wide (front to back) and 4¼ ft. high with a water depth of about 3¼ ft. Their lengths, in order from the west end, are 10½, 10, 15¼ ft. and six tanks averaging about 5 ft. each. The viewing panels or openings of the four cast-iron frames holding the glass fronts are all about 4 ft. 7½ in. long × 3 ft. high. The first two tanks have two viewing panes each (one frame holding all four), the third three (one frame). The plate-glass panes are all $1\frac{1}{16}$ in. thick and the slate backs, sides and bottoms are 1½ in. thick. The slate bottoms of these tanks are 2¼ ft. above the tank-room floor. The lower edges of the viewing panels are 29½ in. above floor-level.

North-Side Tanks

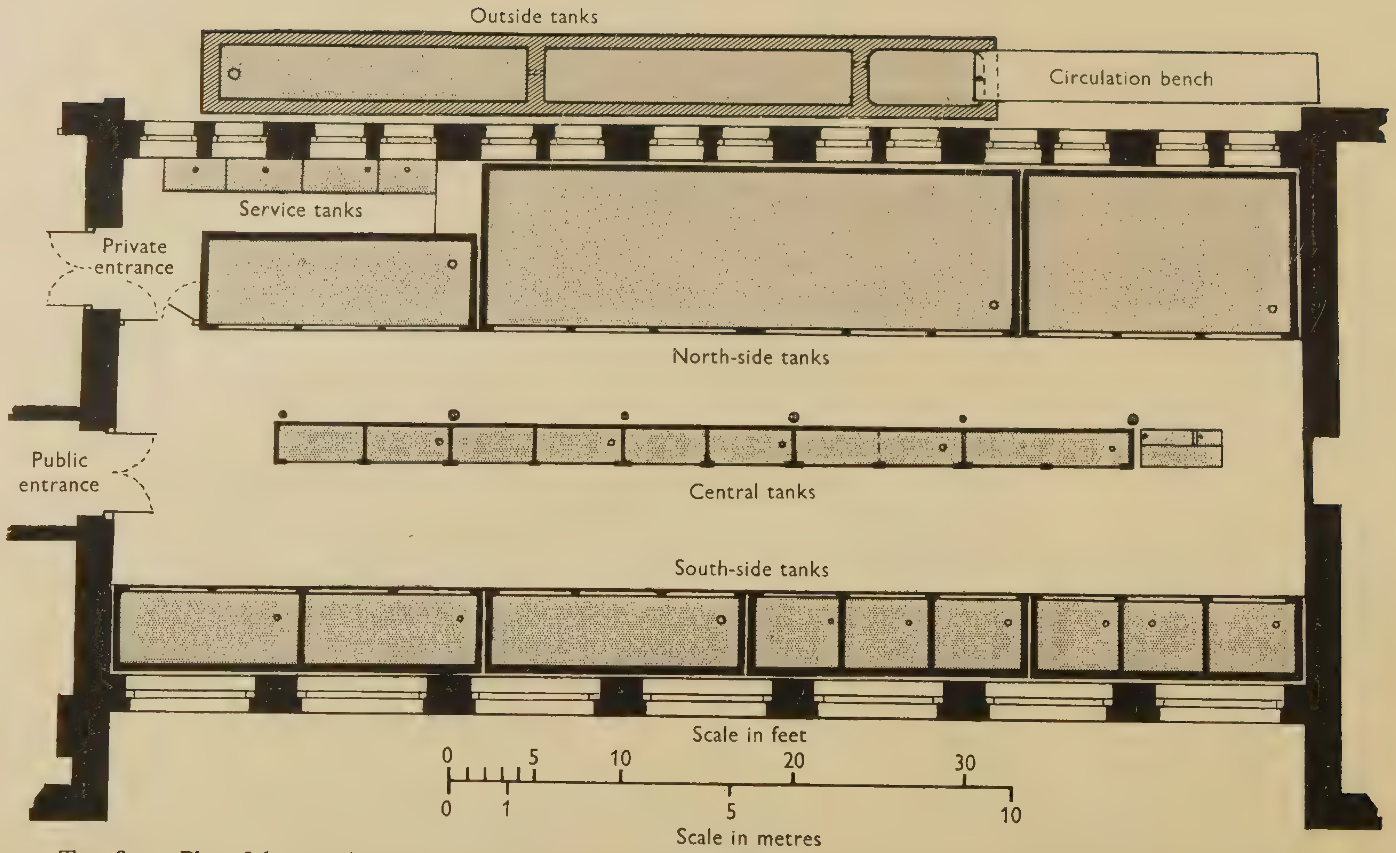
On the north side of the aquarium there are three large tanks, all about 5¼ ft. high with a water depth of about 4¼ ft. Their lengths and widths, in order from the east end, are 15¾ × 9, 31 × 9 and 15¾ × 5 ft. The first two back against the north wall, but the last has a 4¼ ft. wide passage between it and the wall; this passage contains four shallow service tanks. A doorway between the west end of this tank and the west wall of the room excludes the public from this passage and serves as a private entrance to the aquarium.

The viewing openings of the four cast-iron frames (each of three openings) holding the glass fronts of these north-side tanks are all about 4 ft. 7½ in. long × 4 ft. high. The two shorter tanks have three glass panes each, the longest six. The plate-glass panes are all 1$\frac{1}{16}$ in. thick and the slate backs, sides and bottoms are 2 in. thick. The internal bottom-level of these tanks is 2 ft. 2 in. above tank-room floor-level. The lower edges of the viewing panels are about 29 in. above floor-level.

Glazing

The plate-glass panes of the north- and south-side tanks are not set in grooves. The cast-iron frames have machined facings which, when a pane is to be set, are covered with a suitable composition. The bottom edge of the pane is placed on two small composition-covered hardwood blocks, one at each end, and is eased into contact with the composition on the facing along the bottom edge of the frame. The whole pane is then raised into the vertical position and pressed home against the composition all round. It is then tightened against the composition with the aid of wooden shores, battens and folding wedges until the composition begins to ooze out all round in front. The tank is now flooded and the rising water pressure forces the pane in more firmly still. Eventually no more composition is squeezed out and battens and boards float to the surface. To ensure that no air pockets remain in the sealing composition a wooden wedge is used to ram in all round as much composition as can be forced between the glass and the facings of the iron frame. A wooden turn-buckle fixed to the top of the frame ensures the safety of the glass should it accidentally be pushed backwards when the water-level in the tank is lowered during cleaning or other operations. When the tank is full the pressure of water is amply sufficient to keep it in place.

For many years the sealing composition was a mixture of white lead (7½ lb.), powdered whiting (1¼ lb.) and russian tallow (½ lb.). This mixture eventually sets hard and may crack away from the glass, giving rise to leaks. When a pane had been set for several months it was no longer advisable to lower the water level, or to empty the tank, without being prepared to reset the pane. More recently the commercial preparation 'Glasticon' (manufactured by Industrial Engineering Ltd., Mellier House, Albemarle Street, London, W. 1) has proved superior; it does not dry hard and therefore does not crack away from glass or frame. It has the additional advantage that should a leak develop (a rare occurrence) caulking can often be undertaken successfully from the *outside*. This preparation is now used for all the tanks and over a period of five years has given no trouble.


Outside tanks
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North-side tanks
Public entrance
Central tanks
South-side tanks
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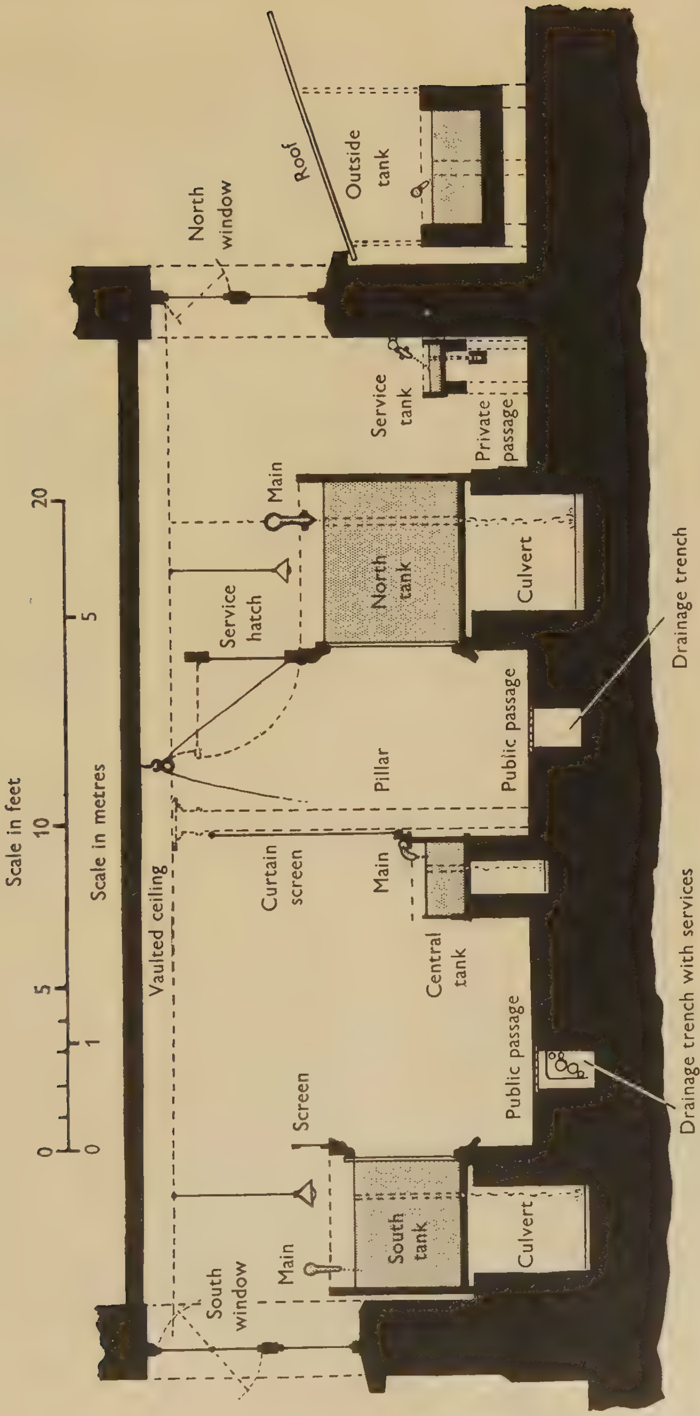
Scale in metres

Text-fig. 1. Plan of the aquarium and outside circulation. Tanks containing sea water stippled. The small circles inside the tanks mark the positions of the overflows.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Vaulted ceiling
North window
Roof
Outside tank
Service tank
Private passage
Main
Service hatch
North tank
Culvert
Drainage trench
Pillar
Public passage
Curtain screen
Main
Central tank
Public passage
Drainage trench with services
Screen
South window
Main
South tank
Culvert

Text-fig. 2. Section through the aquarium near the west end. Sea water stippled.

Central Pillars and Screen

Down the middle of the tank room a row of six iron or steel pillars support the vaulted ceiling and the first floor of the laboratory. These are centrally placed between the outside walls of the building, but because the north side tanks are wider than those on the south side they appear to be sited asymmetrically. A screen or curtain of dark painted fabric is hung between these pillars to prevent the reflexion in the north-side glass panes of the south-side windows which are visible over the tops of the south-side tanks.

Central Table Tanks

Backing up against the pillars and the curtain is a series of five slate table tanks with glass fronts facing south. They are nearly 10 ft. long and 2¼ ft. wide and have a height of 1½ ft. at the front, being some inches higher at the back and sides. The top edges of the ten ½ in. plate-glass panes held in grooves in the slate are rounded and polished, and are only 3½ ft. above floor-level. An adult is thus able to bend over the tank and inspect the contents from above, in addition to viewing them from the front if he stoops down. The water-level in these tanks varies from 8 to 15 in. according to the nature of the display. Most of the tanks are divided into two by a central partition. At the east end of the series a shallow wooden table tank has been added and above it are two small wooden tanks with glass fronts and backs so that they may be viewed from both sides. There is also a small glass-fronted slate tank above the central table tanks near the middle of the series. This is seen in the photograph in Pl. I, but has not been indicated in the plan (Text-fig. 1.)

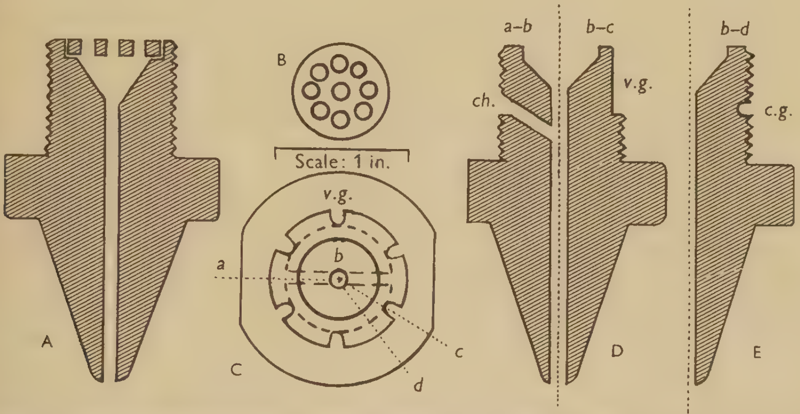
Designers of new aquaria should note that open table tanks are not entirely satisfactory for public display; they are a temptation to boys and some adults to interfere with their contents.

Culverts

The tanks are all built over culverts (originally termed 'circulating reservoirs') which receive the overflow water and return it to the reservoirs. The culverts on the north and south sides are about 3 ft. 9 in. high and almost as wide as the tanks above. Entry to the south-side culvert is through the floor of the westernmost tank; it is normally covered by a large slate sealed around the edges. This is an unsatisfactory arrangement, for the culvert can only be entered when this tank is drained. The north-side culvert can be entered by the west end of the largest tank at any time; the culvert of the central table tanks cannot be inspected at all. Under the two largest tanks on the north side a series of 9 in. brick pillars are staggered down the centre of the very wide culvert to support the weight of the tank floor near the middle. All culverts are lined with asphalt.

flow nozzles

Sea water pumped from the reservoirs enters the main tanks through a ries of vulcanite nozzles situated generally towards the backs of the tanks and few inches above water-level. Some nozzles are controlled by stopcocks. he smallest tanks are each supplied with two nozzles, the others with more ccording to size. Two nozzles are an absolute minimum for safety, owing to ne risk of occasional blockage by foreign bodies. A blocked nozzle may not e noticed for hours, especially during the night, and there is a real danger f fishes in a tank dying before the absence of circulation to that tank is oticed.



ext-fig. 3. Diagrams of vulcanite nozzles modified to delay blockage by debris. A, vertical section of a nozzle provided with a perforated Perspex plate, shown in plan at B. Another method of modification, devised by Mr A. N. Bennett, is shown at C–E. C, plan from above; D, sections *a–b* and *b–c* as indicated on C. E, section *b–d* indicated on C. Six vertical grooves, or channels, *v.g.*, are cut through the screw thread to conduct water to a circular groove, *c.g.* From opposite sides of this circular groove are bored two downwardly sloping holes or channel-ways, *ch.*, to tap the central bore of the nozzle. Until the entrances to the central bore and all six vertical channels are blocked the nozzle continues to function.

Blockage of the nozzle is caused generally by pieces of sponges (*Hali-* *hondria bowerbanki* and *Sycon coronatum*) breaking off from growths in the ipes. This is a constant source of trouble. Most nozzles are now provided vith a perforated disk fitted internally across the full bore of the pipe Text-fig. 3 A, B), or have several channel-ways cut so that all channel- vays have to become blocked before the nozzle ceases to function (Text- g. 3 C–E). This latter method, devised by Mr A. N. Bennett, the engineer in harge of pumps and pipes, has proved very satisfactory.

The water passing through a nozzle is forced down into the tank as a strong et, often carrying with it an inverted fountain of bubbles. It is certain that nly a fraction of this water reaches the bottom, much of it being quickly ost down the overflow, which draws off surface water. To lessen this loss of newly injected circulation water there have in most tanks been placed one or more pipes of 3–4 in. bore, hung vertically from just above surface-level to within a few inches of the bottom. Water from one or more nearby inflow nozzles is conducted by rubber tubing into the top of each such pipe which ensures that the whole of it is led to the bottom. This arrangement has improved the health of the inhabitants of all tanks where it is in use. In the central table tanks glazed earthenware pipes, painted with black bitumastic paint, serve a similar purpose and keep the surface free from the ripples which, before they were used, obscured much of the view.

Overflow pipes

Each of the tanks has a single overflow pipe at one side and usually to the front. This is a vulcanite pipe of bore varying from 1 to 4 in. according to the tank. Its lower end is tapered and fits into a vulcanite seating, tapered to receive it, in the bottom of the tank. To empty a tank the overflow pipe is simply pulled up and the water rushes out through the hole thus opened in the tank floor. Overflowing water drains into the culvert under the tanks and is thereby returned to the reservoir in use, flowing into it at the surface against the north wall. The depth of water in the wide airy culverts (there are ventilators to the culverts through their walls from the outside of the building) increases from a fraction of an inch to 1 or 2 in. towards the reservoir end; good aeration therefore takes place before the water reaches the reservoir.

To obviate flooding in the event of an overflow pipe becoming blocked there are perforated vulcanite grids let into the slate sides of the tanks just above water-level. A stoppage of the overflow pipe results in a slight rise in the water-level of the tank which then overflows into its neighbours on either side.

Drainage Trenches

The floor of the aquarium in the public gallery contains two trenches 18 in. wide and 18 in. to 2 ft. deep, running the whole length of the tank room about 2 ft. in front of the north and south tanks. They are covered by iron gratings level with the rest of the floor, which is of concrete. In addition to containing various service pipes for the laboratory they function as drains running to waste and will carry away water overflowing on to the floor. They are useful when a tank is cleaned by siphoning, as described below (p. 206).

Lighting

Tanks are normally lit only by daylight through the windows. The tanks of the south side receive most light, but direct sunlight is now diffused by ground glass fitted into the south-side window frames. Formerly, when the windows were of clear glass, narrow sunbeams passed through the tanks and their dazzling brilliance made it difficult to see anything outside their range.

n the north side the lighting is always diffused and, except on bright days, insufficient to light the tanks adequately. The smallest tank on this side is pecially dark for, owing to the passage behind it, direct light from the ndows hardly penetrates into it and what it does get is mainly reflected from e white ceiling above. Various devices have been tried to improve the ghting of this tank; at the present time independent electric lighting by ree 60 W. daylight bulbs raises the intensity of the illumination to near at of the largest tank next to it, and is proving more satisfactory than flectors.

The top front edge of the row of south-side tanks is only $6\frac{1}{4}$ ft. above floor-vel and before the war the eyes of visitors were dazzled by direct light from e sky seen through the high arched windows behind the tanks. If the eyes ere shielded with the hand, or the rim of a hat, visibility into the tanks was eatly increased, details not before visible becoming apparent. Therefore, uring the reconstruction after the war, a 14 in. high plywood screen was ected along this top front edge so that the tops of the windows could no nger be seen from a normal viewing position. It was not possible to carry his screen close up to the ceiling (as was done on the north side) on account f the central table tanks. These are lit only by light passing over the screens be reflected from the white ceiling above them. The screen is painted white n the window side to reflect light on to the backs of the tanks which formerly ere barely visible. The screen is easily removable in sections to facilitate iphoning or other work.

The north-side screen almost reaches the ceiling, leaving a narrow gap r ventilation. At intervals there are hatchways for feeding, glass-cleaning nd similar purposes. If ever the central table tanks are removed it would be dvantageous to treat the south side in the same way, namely to extend the resent low screen to near the ceiling and to provide appropriate hatchways. f this were done the central screen between the pillars would no longer be eeded and the aquarium would become a fine hall lit only by light passing hrough the tanks.

In addition to the special electric lighting of the dark tank on the north ide, electric lighting has recently been installed over all tanks (except the able tanks) for use on dull days, or after dark. Ordinary 60 W. pearl bulbs not daylight type) in white plastic reflectors, approximately one to every lass pane, give very good illumination; they hang a few inches above water-evel towards the fronts of the tanks. The general appearance of the tanks fter darkness with these lights on is better than their appearance by day ithout them. This is probably due almost entirely to the direction of the ght, coming from the top front of the tank instead of from the back. By day he side of the fish away from the observer is more brightly illuminated than he side which is on view. Only a few semi-transparent organisms are nhanced by back-lighting in the absence of frontal lighting.

Decorative Rockwork

To relieve the bare appearance of the slate tanks, and to improve living conditions for the inhabitants, most of the large tanks have rockwork built into them. Some of the original rockwork, dating from the time the tanks were first erected, can be seen in the largest tank on the north side. This original rockwork consists of lumps of calcite stuck together and to the slate walls with pitch. No attempt was made to achieve a natural effect. In 1946, when war damage to the aquarium was repaired, new rockwork was added to several of the tanks and much of the old rockwork reinforced with concrete. For the new rockwork, which was built by our own staff, waterworn limestone was obtained from the foreshore at Cattedown (district of Plymouth) and care was taken to arrange and cement the slabs to give an appearance of natural stratification and to provide suitably shaped holes for octopus, lobsters, conger eels, etc. In one tank stalagmitic rocks have been used with good effect. The floors of the tanks are covered with sand or shell gravel or with water-worn pebbles in accordance with the species kept in them. Wherever slate backs and sides are uncovered by rockwork they are painted over with 'Bituros', a black bituminous paint used for drinking-water tanks and manufactured by Wailes-Dove Bitumastic Ltd., Hebburn, Durham.

Labelling

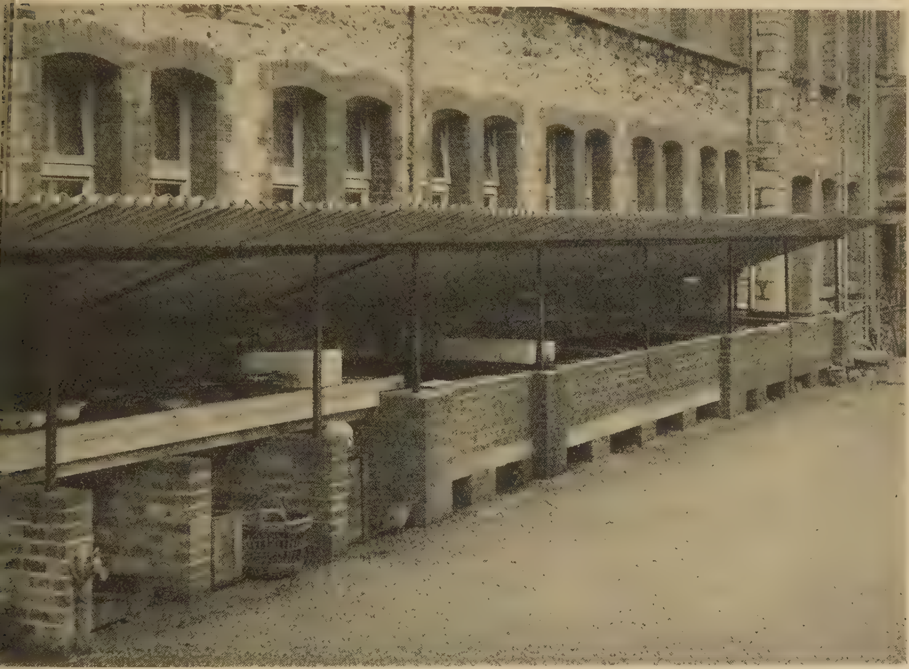
Lead frames containing labels are screwed to moulded wooden sills on the north- and south-side tanks. Name labels are constructed by pasting a printed paper sheet face down on to glass and backing with a sheet of white opal glass stuck on with paraffin wax. Picture labels are painted and lettered in water colour on good quality drawing paper and sandwiched between thin sheets of Perspex sealed around the edges. This is undertaken by a firm specializing in the process. Such Perspex labels should be permanently waterproof.

OUTSIDE CIRCULATION

In the yard between north and south laboratory buildings there are three asphalt-lined brick and concrete storage and acclimatization tanks and a circulation bench for bowls (Text-fig. 4). They are built against the north wall of the south building and are screened from sky and rain by a pitched roof of corrugated asbestos but are otherwise open to the outside air. Two of these measure internally approximately 18 ft. 6 in. × 3 ft. 6 in., the water depth being 1 ft. 6 in.; they can be divided into smaller compartments by movable wooden partitions. The third tank is only 7 ft. long, but is similar in width and depth. Water is supplied by a series of jets from an iron pipe. Although very rusty, this pipe has already given adequate service for several years.

Laboratory Tanks

In the main laboratory on the first floor are tanks of various sizes for storage and research. There are eight rustless-steel framed tanks with 1 in.-thick slate bottoms and sides and $\frac{1}{2}$ in.-thick plate-glass fronts. The three largest measure 4 ft. × 2 ft. 4 in. × 1 ft. 6 in. deep externally. There are also sixteen porcelain sinks, 2 ft. × 1 ft. 6 in. external measurements, eight 10 in. deep externally, four 8 in. deep and four 6 in. deep. Two shallow table tanks, made from $1\frac{1}{2}$ in. thick teak measure externally 7 ft. × 5 ft. × 9 in. and 5 ft. × 3 ft. × 9 in. The water depth is only $6\frac{1}{2}$ in. With such a relatively great surface area one or two small nozzles suffice to keep a great variety of small animals alive. These tanks are indeed among the most successful for small organisms. Such tanks are unfortunately not suitable for public display owing to the ease of access to their contents.



Text-fig. 4. View of the outside circulation showing portion of circulation bench and the three asphalt-lined brick and concrete tanks. The two sink-tanks are temporary additions.

Most of the tanks just described can be seen in photographs reproduced by Russell (1948, plate XIX). In these photographs it will be observed that provision is also made for giving circulation to bowls, etc., placed on slate circulation benches.

Overflow water from these laboratory tanks and from the circulation benches runs directly into exhibition tanks in the public aquarium on the ground floor below. This is a bad arrangement, because from time to time silty water produced by activities in the laboratory clouds the exhibition tanks into which it is discharged and adds considerably to the labour of siphoning needed to keep them reasonably clean.

In conjunction with the Specimen Supply Department in the north building there are four sink tanks and a small circulation bench. Formerly this had its own separate pump to supply water from one of the reservoirs, but of recent years an iron pipeline has been installed to supply it direct from the main pump.

The Reservoirs

Sea water is pumped into the tanks from one of two large reservoirs, each 37 ft. × 21½ ft., holding water 11 ft. deep. They are used alternately. The reservoirs were excavated in the solid limestone north of the aquarium at the time the latter was built; they were completed with concrete and lined with asphalt. They lie below general ground-level and are roofed over with a concrete flat to shield them from light and rain. Each holds about 55,000 gallons, or about two and a half times the total volume of water in the tanks, including those in the main laboratory on the first floor and the large specimen storage tanks in the yard outside.

Overflow water from the aquarium is discharged into the reservoir in use at the surface in a corner by the north wall. The outflow to the pumps is sited about 3 ft. from the bottom not far from a corner on the south wall. A complicated swirl is set up throughout the reservoir and there is no completely stagnant corner. In some parts the water moves extremely slowly; in other places and at different levels it moves much faster (determined by drift-bottles). It seems possible that some of the water returned from the aquarium is drawn off again to the pumps before water which has been in the reservoir for a longer time. The swirl must hinder the settlement of slowly sinking detritus brought from the tanks and much of this detritus is pumped back into the tanks again (see p. 209 for a suggested method of dealing with this problem).

The Engine Room

The engine room is in the basement and its floor is about level with that of the reservoirs. Circulation is maintained by one of two centrifugal pumps driven by electric motors. The older pump is a cast-iron 2 in. pump driven by a 3 h.p. motor; the newer one is a cast-iron 2½ in. pump driven by a 2 h.p. motor. Water drawn from one or other of the reservoirs at a point in the south wall about 3 ft. above the bottom is pumped at over 50 gallons a minute against a head of about 40 ft. through 2 in. cast-iron glass-lined piping to the main vulcanite pipes serving the tanks.

The pump impellers are of cast-iron mounted on stainless-steel spindles. A cast-iron 2 in. centrifugal pump driven from shafting by a 5 h.p. Crossley gas engine is a stand-by in the event of electrical failure. Except for two periods of about 1½ hr. in the morning and for ½ hr. in the evening, for cooling motors and for maintenance, pumping continues day and night.

The engine room also contains two air-compressors, but of recent years compressed air has had little use in the tank room though it is supplied to, and has its uses throughout, the laboratory.

PUMP HOUSE

Reservoirs are periodically emptied, hosed down, and refilled from the sea. For this purpose there is housed in a small brick and concrete building on the rocks just above sea-level, which is about 97 ft. below laboratory ground-level, a cast-iron 4 in. centrifugal pump driven by a 14 h.p. motor. This is capable of delivering about 215 gallons a minute against a head of 150 ft. and can fill a reservoir in about 4 hr. A good spring tide in calm dry weather is chosen, and pumping commences 1–2 hr. before high water and continues for about the same time afterwards. Usually part of the volume required is pumped on one tide and the remainder the next day. The water is drawn through a suction rose situated near low-water mark at the base of the rock on which the pump house is built. The Shone's Ejector mentioned by Allen & Harvey (1928) was dismantled years ago, as was the last of the 'Otto' gas engines.

MAINTENANCE

Treatment of the Stored Sea Water

During the summer one reservoir may be emptied, cleaned and refilled every month, but during the winter several months may go by without new water being obtained from the sea. Sometimes reservoirs are merely lowered a few feet and then filled up. In normal practice a reservoir is in use for 1 week, while the other rests. At the end of the week there is a change over. The reservoir passing out of use is then limed, that is to say, a bucket is one-third filled with quicklime and slaked under a freshwater tap. The slaked lime is spread as evenly as possible over the reservoir and sinking through the water restores the pH to that of natural sea water. It has been found advantageous to treat (with slaked lime) the reservoir actually in use about the fourth day, especially during hot weather. Water newly drawn from the sea often decreases in pH more quickly than old established water which has been frequently limed. This limed water is very satisfactory for ordinary aquarium purposes. For notes on the chemistry see Atkins (1931) and Cooper (1932).

Temperature

There is no means of artificially heating or cooling the aquarium water, its temperature varying with the seasons. The upper and lower limits are roughly 17–18° C. in summer and 7–8° C. in winter, occasionally being exceeded in both directions. For the fauna kept in our tanks temperatures above 16° C. and below 9° C. are undesirable. Quite a number of species suffer when the temperature changes rapidly (2–3° C. in a week), especially near the upper and lower limits.

The water flowing through the outside circulation bench and tanks, exposed to the outside air, is almost invariably cooled down and returned to the reservoir at a lower temperature than that from the rest of the system, the general effect being to cool the whole. Very rarely in summer the reverse may happen. In hard frosty weather it has sometimes been necessary to cut off this outside circulation to conserve the heat of the main system.

Salinity

Few data on the salinity of the aquarium water are available. The salinity of the water pumped at high tide is about 35 ‰, but increases somewhat with storage; for instance Cooper (1932) mentions a figure as high as 38·0 ‰, and Brown (1929) had previously given figures 37·0–37·9 ‰. It has never been necessary to add fresh water to make up for that lost by evaporation, and no effects on the animals attributable to increased salinity have been noted.

Servicing the Large Tanks

There are no service galleries behind the large north- and south-side tanks in the Tank Room and all work, such as feeding, glass cleaning and siphoning has to be carried out over the front of the tanks from a ladder propped up against them. On the south side the attendant can make his way along the backs of the tanks via the internal window sills, on the north side along an inconvenient cat-walk of planks placed across girders just above water-level (the two largest tanks only). There is insufficient head room to walk upright on this cat-walk.

Glasses are cleaned once or twice a week internally with a scrubbing brush attached to the end of a pole, or with a straight edge of Perspex on a long handle. This latter will remove quite hard growths without scratching the glass. Old newspaper serves for the exterior of the panes.

Silt which accumulates on the bottom of the tanks is removed by siphoning with a rubber hose, the siphoned water running to waste through the drainage trenches in the aquarium floor. Sand or fine gravel brought over from the tank is trapped in a galvanized iron bath into which the siphoned water first discharges. It is sometimes useful to fit a large funnel on to the end of the hose inside the tank. By choosing one of the right size flocculent silt may be arated from fine gravel. A tap on the outer end of the hose, to regulate of water flow, can be of assistance in this.

ding

The main food used in the aquarium is the common squid, *Loligo forbesi*, ainable at Plymouth in quantity most of the year round. The uncooked ite mantle flesh of this animal, cut into suitable sized pieces, is relished by ost all the species kept, while the heads are eaten by the conger eels and rsehounds. The contents of ovaries dispersed in the water have proved ellent for feeding very small fishes such as young grey mullet. At times, hen squid is not to be had, herring, mackerel, conger eel and some white hes are used. The oily fishes tend to foul the water and the white fishes are relished and are generally not eaten at all unless really fresh. Iced fish tained from the fishmonger is often left uneaten by hungry fishes. Worms *reis diversicolor*) are also an excellent food for smaller fishes.

A few animals need a specialized diet. Octopuses and cuttlefishes must be pplied with living crabs or prawns, although they can occasionally be duced to take dead fishes. John Dories generally require whole fishes but be trained to take squid, especially if it be cut into an elongate shape. pefishes need living plankton. The overflow from a main laboratory tank ds into the pipefish tank and jars of living plankton, after satisfying the eds of workers in the main laboratory, are tipped directly down this over- w. Most fishes need to be fed at least twice a week, but daily is too often. ccessful feeding demands care and attention to the varied ways of catching ey natural to the different species kept. A complete account would occupy eral pages.

General Remarks and Suggestions

uring the last few years a number of plans for proposed new aquaria have en submitted for criticism. A common fault has been that the sizes of oposed tanks have been too small, especially widths in relation to lengths. cept for quite small fishes a width of 3 ft. (commonly adopted) is insufficient, pecially when it is proposed to fix rockwork inside on the back. Quite apart om matters concerning the health of the fish it is often forgotten that a tank ll of water viewed through the glass appears to be considerably less wide an it actually is. While for viewing small organisms lying at the back of the nk this may even be an advantage, an aquarium consisting solely of parently narrow tanks fails to give that illusion of an underwater world hich is one of the charms of a properly designed aquarium.

There is no doubt, too, that large tanks are better for their inhabitants and ve less trouble than do small tanks. In general the smaller the tank the ore often does it require attention and the more likely are the results of temporary stoppage of circulation to prove fatal. Some small animals, vertebrate and invertebrate, which have never done well in our smaller tanks have flourished and lived a full natural life in our larger ones. Thus the little Two-spot Goby (*Gobius flavescens*), which never lived for long in small tanks, does extremely well in the largest tank of all. Presumably in a very large tank the goby readily finds scraps of food left over by the big fishes, as well as small worms, crustaceans, etc.; it is itself too small for the big fishes to eat. A great improvement in health and vigour has often been noted when fishes such as pollack, whiting, red mullet and sea-bream have been transferred from tanks on the south side to north-side tanks with several times the capacity.

A tank floor need not be level; it could be constructed to slope downwards from the front towards the back. This would give increased depth (at the back) without undue pressure on the glass. It may also slope upwards when it is desired to construct a series of rising terraces for anemones, sea-fans and other sedentary organisms.

Another point is the size of reservoirs. In a closed circulation the bigger the reservoir in relation to the inhabited tanks the better. During the war when most of our big tanks were broken and empty, the inhabitants of those which remained did noticeably better, and delicate organisms survived longer than they did before the war or do now, and sometimes even bred. As the capacity of one of our reservoirs is roughly two and a half times the capacity of the tanks it seems reasonable to conclude that a reservoir capacity double or treble this would give noticeably improved results. The relative proportions needed are influenced, of course, by the density of stocking, by liming, filtration and other treatments of the water, but there seems little reason to doubt that in designing a closed circulation aquarium it is advisable to construct the reservoirs on as generous a scale as possible. Such reservoirs must be darkened to inhibit the growth of water-clouding phytoplankton organisms; they should not be tanks exposed to the atmosphere and thereby able to gain or lose heat relatively rapidly. In the same way exhibition tanks should not be exposed to the sun or directly to the outside air; the whole system should be enclosed in a building. If the reservoirs be well insulated and if the building itself be centrally heated there should be little difficulty, in our climate, of maintaining a reasonable temperature during the winter months.

No public aquarium should be designed without service galleries behind the tanks. Such galleries not only greatly ease and expedite attention to inflows and overflows and all work of feeding, cleaning, addition and removal of specimens, but their lack can lead to difficulties on crowded days when it becomes scarcely practicable to give emergency attention from a ladder over the front of a tank. If during a Bank Holiday anything goes wrong it may not be possible for several hours to do anything to put it right.

Similarly, all aquaria should be provided behind the scenes with tanks for acclimatization and excess stock, living food and for sick fishes. It is bad ctice to put freshly caught fishes into the exhibition tanks. Even those t will survive often show bruises, torn fins and other injuries during the t 2 or 3 weeks and a damaged fish always attracts attention. Acclimatization ks must be large and deep, otherwise they will fail in their purpose. Storage ks and tanks for sick fishes can be appreciably smaller. In cases of infectious kness the overflow water should run to waste; it may be wiser not to attempt keep the fish alive at all.

A frequent inquiry concerns filtration; should the water be filtered? Many ll-known aquaria do possess elaborate filter beds and the water in their nks is crystal clear. This condition seems to suit most fishes, but some vertebrates, and especially filter-feeding invertebrates, do not seem to do well. The filter-feeders could be provided with suitable cultures of microganisms, but on the whole for them the water is better not filtered. It ould, however, be allowed to sediment while passing through the reservoir. is could be achieved by partitioning the reservoir so that it becomes in ect a long broad passage way, repeatedly bent back upon itself, through ich the water would flow slowly from inflow to outflow. Overflow water m the aquarium would be conducted through a very broad pipe, or shaft, aight to the bottom at one end of the passage, and water to the pumps uld be drawn off above the bottom at the far end. During the hours the ter would take to traverse the passage sediment would be deposited on the ttom. Sedimentation would remove unwanted detritus without removing mming micro-organisms.

A disadvantage of encouraging filter-feeders is the likelihood of sponges d other organisms growing in the pipes through which the sea water is mped to the tanks. The blockage of nozzles from pieces of sponge has eady been mentioned (p. 199). The trouble would not be so great if pipes re provided with some easy means of access at all bends and elbows; ernal growths could than be scraped off from time to time.

In a permanent installation pipes conveying water under pressure need to made of vulcanite or some other material not affected by sea water. This plies equally, of course, to all parts in contact with the circulating sea water. pper, brass, zinc and galvanized iron are the metals most likely to be countered in constructors' plans. In sea water they dissolve to a sufficient tent to poison marine organisms. Pure lead is permissible in small quantity t should be avoided if possible. In our experience vulcanite pipes and cks have proved perfectly satisfactory; some of the original ones are still use after sixty years of service. On the other hand, stainless, or rustless el pipes installed to supply new tanks erected in the main laboratory during construction in 1938–9 rusted through, especially at bends, in only a few ars, though stainless-steel has been satisfactory for the outside framework small slate and glass tanks and for pump spindles. Ordinary iron piping, spite of extensive rusting (which is harmless) has given better service and is very satisfactory for a temporary installation which is expected to las only for a few years. Internal rust must be scraped out from time to time Rubber hose can also be used for temporary erections. Some of the newe plastic materials may prove excellent for sea-water aquarium pipes but w have had no experience with them. Pipes of transparent or translucen materials would need to be darkened to prevent algal growths inside.

For overflow water not under pressure ordinary earthenware drain piping or asbestos pipes or troughs may be used.

Finally, there is the appearance of the tanks to be considered. The ai should be to present the animals in surroundings as natural to their specie as is possible; this is not only good showmanship but is also beneficial to th animals. Carefully chosen natural rocks placed in position with due regard t their normal geological formation, sand or gravel or pebbles on the botto (not too deep a layer), whatever is suitable, can greatly enhance the attractiveness of the display, but over-elaboration should be avoided. It is not desirabl that the rockwork should attract the eye away from the fish. All inflows an overflows should be concealed. Inflows, of which even the smallest tank should have at least two, can be placed in the forward corners of the tank behind rockwork or anywhere else out of sight. Inflow nozzles should discharge into wide pipes passing from just above surface-level to near th bottom (see pp. 199–200) to ensure good circulation throughout the tank Overflow water should pass through a hole in the back of the tank and shoul draw off surface scum. There is no need for unsightly pipes stuck into seating on the tank floor.

Tanks can always be emptied by siphoning, but a plug could be provide in the tank floor in case of need. Aeration by compressed air forced throug pieces of cane, or through diffusers manufactured for the purpose, should n generally be required, but when the temperature is high can be used wit advantage. One use is to assist circulation in the tank by creating an upwar current from the bottom. This is most effective if the air be bubbled up throug a wide tube opening near the bottom and just below the surface. It should b noted that water supersaturated with air, or containing an excess of fine ai bubbles, may not be good for the health of the fish. It may be a cause of th gas blisters which from time to time occur in the eyes, fins and skin of som fishes.

Excess daylight must be avoided if too vigorous a growth of algae is to b prevented. A slow growth of small red weeds can be permitted and will occu if the amount of daylight allowed is not reduced too much in intensit Electric lighting for dull days and at night should be fitted, especial care bein taken to ensure perfect insulation and water-proofing. Lights should b situated well forward (see p. 201), and close to the water surface, but it i a matter for choice whether fluorescent tubes or ordinary bulbs are used. Th former give a shadowless light, the latter throw shadows and when the surfac the water is rippled there is produced a play of light on rocks and sand hich adds liveliness to the appearance of the tank.

In designing the layout of an aquarium care is necessary to ensure that no ight reflexions obscure the view. Windows, doorways, brightly lit tanks pposite, and even white labels reflected in the glass can distract from and bscure the tank contents. All paint-work should be very dark, for the same eason.

The slate backs and sides of the Plymouth tanks where not covered by ockwork are painted black (see p. 202). This is not always ideal. The system dopted at the Danish Aquarium at Charlottenlund has much to recommend . Tanks are lined with various blue and green toned semi-opaque or enamelled lass, enhancing the brilliance of the tank, and showing off some fishes to etter advantage than does black. A system of false backing, a sheet of ground r semi-transparent glass, perhaps lightly coloured, placed a few inches in ront of the real back of the tank gives an impression of distance, especially fish can penetrate behind the false back to be dimly seen. The edges of the alse back must be concealed.

There are no guard-rails at Plymouth and on crowded days people crush p against the glass and hide the labels from view. A leaning rail in front of e tanks should always be provided, as it not only protects the glass and labels ut is a definite comfort to the visitor.

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EXPLANATION OF PLATE I

The aquarium viewed from a height of 7 ft. close to the wall at the east end. Left to right: south-side tanks with low anti-dazzle screen along front top edge; cast-iron gratings covering drainage trench in floor; central table tanks with small glass-fronted slate and wooden tanks above; six central pillars with curtain screen hung on them (three sections of the screen near to the camera have been removed); north-side tanks with service hatch in screen open and service ladder in position. The public entrance, closed by double doors, is at the far end. The pipes crossing the ceiling in the middle distance are sea-water mains and overflows to and from tanks in the main laboratory above. Some details of the electric lighting to the tanks are also visible.

ABSTRACTS OF MEMOIRS

ECORDING WORK DONE AT THE PLYMOUTH LABORATORY

The Onset of Shortening in Striated Muscle

By **B. C Abbott** and **J. M. Ritchie**

Journ. Physiol., Vol. 113, 1951, pp. 336–45

hen a striated muscle is stimulated at one point, the excitation travels away om that point at a finite speed. The whole of the muscle is active when the ave of contraction has travelled the length of the muscle. Doubt has been st as to whether the electrical wave associated with membrane excitation avels at the same velocity as the wave of contraction.

Isolated muscles from dogfish, frog and toad at 0° C. were stimulated) simultaneously at many points along their length, and (*b*) at one end only. he time course of unloaded isotonic shortening during maximal twitches as recorded photographically. When simultaneously stimulated all over the uscles began to shorten at their maximum speed at the end of a latent period. hen stimulated at one end the speed built up gradually, but to the same aximum value, so that the shortening-time curves run parallel: the curves e separated in time by half the propagation time of the contraction wave ong the muscle. It is shown experimentally that in the muscles of all three imals studied the propagation velocities of contraction and excitation waves e identical. When a muscle is stimulated in saline the propagation velocity appreciably greater than when in air. B.C.A.

he Localization and Analysis of the Responses to Vibration from the Isolated Elasmobranch Labyrinth. A Contribution to the Problem of the Evolution of Hearing in Vertebrates

By **O. Lowenstein** and **T. D. M. Roberts**

Journ. Physiol., Vol. 114, 1951, pp. 471–89

ibration responses in the form of impulse discharges can be recorded from erve twigs leading from part of the macula sacculi, the macula neglecta, and he lacinia of the macula utriculi of the isolated elasmobranch labyrinth. The tolith-bearing part of the macula utriculi, the posterior portion of the macula acculi and the adjoining macula lagenae do not respond to vibrational timuli. They contain gravity receptors only. An appreciable number of the sense endings show a resting activity in the absence of vibrational stimulation There exists, however, convincing evidence that, at any given time, man sensory units are quiescent. These can be recruited to take part in th vibrational responses, and they show a considerable range of thresholds Under the obtaining experimental conditions vibration responses wer recorded to stimulus frequencies extending rarely higher than 120 cyc./sec Vestibular microphonics were observed up to a signal frequency of 750 cyc./sec but only responses in the form of nerve impulse discharges are accepted a evidence for vibration sensitivity. At low intensity stimulation the respons consists of an increase in the discharge frequency of the 'spontaneously firing units. Higher intensities lead to the recruitment of previously quiescen sense endings and to a marked synchronization of the response frequenc with that of the stimulus. This synchronization closely resembles the response described for the mammalian cochlea where it occurs at the lower end of th audible spectrum. Adaptation to sustained vibrational stimulation and 'silent period' after cessation of prolonged stimuli have been observed an the latter has been quantitatively analysed. It is claimed that the theoretica implications of these results may be of considerable importance in relatio to the problems of the evolution of hearing and pitch discrimination i vertebrates. O.L

THE LIFE-HISTORY OF THE MULTIFORM SPECIES *JASSA FALCATA* (MONTAGU) (CRUSTACEA AMPHIPODA) WITH A REVIEW OF THE BIBLIOGRAPHY OF THE SPECIES

By **E. W. Sexton** and **D. M. Reid**

Journ. Linn. Soc. London (*Zool.*), Vol. XLII, 1951, pp. 29–91

It has been shown by rearing and breeding experiments that the amphipo *Jassa falcata* (Montagu 1808) is a polymorphic species.

The species falls into two main classes or divisions, characterized particularl by the differing setation and shape of the second antennae, and the secon gnathopods of the males. These divisions are called here, in accordance wit their appearance, the Broad and the Narrow Forms. There is a third Form, i which the antennal characters of both the other Forms are combined wit either the Broad gnathopod or the Narrow one.

In addition a number of Minor variants occur within the limits of the tw main divisions.

The species also shows male intersexuality.

The synonyms of the species are summarized, and a full review of th bibliography given.

A PHOTOGRAPHIC SURVEY OF CERTAIN AREAS OF SEA FLOOR NEAR PLYMOUTH

By H. G. Vevers, M.A., D.Phil.
Zoologist at the Plymouth Laboratory

(Plate I and Text-figs. 1–2)

INTRODUCTION

The present work is a continuation of that carried out in the period July 949–October 1950 (Vevers, 1951). The underwater photographic apparatus as been modified and improved; the main changes are (*a*) the lamps are now arried on a tubular metal semicircle instead of on a ring of strip metal,) the bottom of the pole is bent at an angle to the main pole, and (*c*) an nproved junction box fixed above the foot switch allows the main electrical able to be detached completely from the remainder of the apparatus. These odifications can be seen in Text-fig. 1.

The apparatus is still capable of taking a series of photographs, each 1 m.^{2} area or less, and in the present work the camera has actually been set at distance of 1 m. from the ground so that each picture covers an area of m.^{2} (50 × 50 cm.). This lower position of the camera is also shown in ext-fig. 1.

There is no doubt that with the camera in this lower position (1 m. from e ground) the definition of the photographs is greatly improved. This may e partly due to a reduction in the absolute amount of suspended particulate atter between the camera lens and the object.

The length of pole above the camera (in its lower position) might appear to e unnecessary, but in practice it has been found that this helps in steadying e whole apparatus when submerged.

In the previous work, using this apparatus, photographs were taken in four istinct areas near Plymouth, namely:

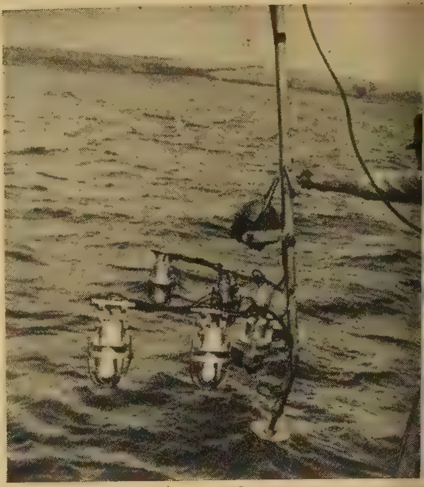
(*a*) Near Station L4, muddy sand with gravel patches, 55 m.
(*b*) South and south-west of Eddystone, 2–4 miles, clean sand, 70 m.
(*c*) South-east of Looe Island, muddy sand and gravel, 50 m.
(*d*) South-west of Looe Island, muddy sand and gravel, 54 m.

No further work has yet been done in areas (*b*) and (*c*), but additional series f photographs have been taken in areas (*a*) and (*d*). Photographs have also een taken in two new areas:

(i) South-west of Rame Head, 2–3 miles.
(ii) North-west of Eddystone, ¾ mile.

The results obtained during the period November 1950 to August 1951 a given below.

1



2

Text-fig. 1. Underwater photographic apparatus hoisted over side of R.V. *Sabella*, show new arrangement of photoflood lamps.

Text-fig. 2. Lowering the apparatus into the water. The camera case is in the lower positi to take a series of $\frac{1}{4}$ m.^{2} photographs of the sea bottom.

AREA SOUTH-WEST OF RAME HEAD, 2–3 MILES, DEPTH 50 M.

A total of 167 photographs, each $\frac{1}{4}$ m.^{2} in area, were taken along the trans Rame Head: 058°T., distance 2·2′; 058°T., 2·8′; 050°T., 3·5′. On t photographs there were 17 empty *Turritella* shells (9 on one and 8 on the othe but there were no living epifaunal animals in any of the pictures. The bott was soft mud, and in many of the photographs the surface showed slight l distinct ripple marks. The area is known locally as the Rame Mud and t characteristic fauna normally dredged there consists almost entirely burrowing forms (*Cucumaria elongata*, *Psammosolen* (*Azor*) *chamasol* *Melinna palmata*). It is therefore not surprising that epifaunal animals shou be more or less absent from this area.

AREA OF STATION L4

The new series of photographs in this area were taken along the follow transects:

24. xi. 50. Penlee Point: 029°T., 4·9′; 033°T., 4·8′; 037°T., 4·8′; 039°T., 5 043°T., 4·7′; 044°T., 4·7′.
15. ii. 51. Penlee Point: 030°T., 4·9′; 035°T., 5·2′; 040°T., 5·2′; 050°T., 3·
26. iv. 51. Looe Island; 310°T., 6·3′; 309°T., 6·6′; 309°T., 6·8′.

Transect of 24 *November* 1950. Six films were taken, of which the first four re very close to one of the previous transects in this area (24 August 1949) evers, 1951, p. 105). These four films showed a uniformly poor fauna, nilar to that already recorded for this area. A detailed count of the epifauna 121 interpreted photographs (covering a total area of 30 m.^{2}) gave the lowing figures: 9 *Ophiura texturata*, 3 *Asterias rubens*, 2 *Marthasterias cialis*, 2 *Chlamys opercularis*, and a single specimen each of a hydroid, urrowing anemone and *Trigla* sp. There were also three distinct tracks, bably made by an animal dragging its body along the surface of the bottom. The first half of the fifth film showed the same type of poor epifauna, but a point about ¼ mile north of the previous transect (24 August 1949) the ture of the fauna changed without any visible corresponding change in the ture of the bottom. In the last half of this film and in the first half of the h film nearly every frame showed crowded *Ophiothrix fragilis*. In thirty- interpreted frames (each ¼ m.^{2}) there were 547 *O. fragilis* and 2 *Ophio*- *ina nigra*, giving a mean density of 70 brittle-stars per m.^{2}. In the second f of the sixth film there were only four frames showing *Ophiothrix fragilis*. six films in this area showed a bottom of muddy sand with gravel, but it is bable that the last few photographs of the sixth were taken near the thern edge of the Rame Mud as they showed patches of bottom with pty shells of *Dentalium* and *Turritella*.

TABLE I

Film	Total of ¼ m.^{2} frames interpreted	Total of ¼ m.^{2} frames with *O. fragilis*	Total number of *O. fragilis*
I	29	—	—
II	23	1	3
III	31	2	16
IV	21	5	35
V	36	27	436
VI	30	—	—

Transect of 15 *February* 1951. Six films were taken, five of them to the th of the transect of 24 August 1949, and the sixth film to the north. Here in, the majority of the 170 ¼ m.^{2} frames interpreted showed only a poor na. The total numbers of epifaunal animals, excluding *Ophiothrix fragilis*, this part of the area were: 6 *Ophiura texturata*, 1 *Marthasterias glacialis*, *Asterias rubens*, 4 *Chlamys opercularis*, 1 *Pecten maximus*, 12 *Eupagurus deauxi*, 1 *Portunus* sp., 1 burrowing anemone and 14 clumps of *Cellaria* sp. is transect has also cut across a dense population of *Ophiothrix fragilis*, ose distribution is given in Table I.

The group of brittle-stars found on this transect is only ¾ mile west of the pulation photographed in the transect of 24 November 1950, and it is bable that both samples belong to the same large aggregation. Both these groups of *O. fragilis* (transects of 24 November 1950 and 15 February 1951 did, in fact, occur in the neighbourhood of the first transect (24 August 1949 photographed in this area, but which did not show any specimens of *O. fragili* It is possible then that the ophiuroid population found in 1950 and 1951 wa new to this locality, but it is more likely that the 1949 transect in this area jus missed it.

Transect of 26 *April* 1951. Three films were taken along this transect whic lay to the north-west of the previous two transects. The bottom was mudd sand with patches of fine gravel (see Pl. 1, fig. 8). The epifauna, as shown b the photographs, was very poor, and the total of eighty-nine frames, representing 22 m.^{2} of the bottom, showed only 7 hydroids, 3 clumps of *Cellari* sp., 4 burrowing anemones, 1 small crab, and 1 narrow track.

Area South of Looe Island

Photographs were taken along two transects:

24. iv. 51. Looe Island: 353° T., 4·8′; 000° T., 5·0′; 005° T., 5·0′; 010° T., 5·2′
25. vii. 51. Looe Island: 000° T., 5·1′; 349° T., 5·1′; 345° T., 5·3′; 341° T., 5·4
342° T., 5·0′.

Transect of 24 *April* 1951. This transect of four films ran from east to we and crossed the previous transect photographed in this area on 2 August 195 The first film showed a bottom of sand and muddy sand and gravel wit a very poor epifauna. On the second and third films the pictures showe a population of *Ophiothrix fragilis* somewhat less dense than that phot graphed on 2 August 1950. The total numbers of animals counted on this pa of the transect (50 ¼ m.^{2} frames) was: 706 *O. fragilis*, 1 *Ophiocomina nigr* 2 *Chlamys opercularis*, and 1 specimen each of *Pecten maximus*, *Marthasteri glacialis*, and a hydroid. The density of *Ophiothrix fragilis* was therefore abo 56 individuals per m.^{2}. The fourth film of this transect showed no aggregatio of brittle-stars except on two frames, and the only other animals record were 3 *Chlamys opercularis* and 1 swimming crab (*Portunus* sp.).

Transect of 25 *July* 1951. This transect of four films ran to the east of tl previous transect, and covered a distance of about 2¼ miles. Nearly eve frame contained a large number of *Ophiothrix fragilis*, denser in numbers th in the previous transect (see Pl. I, figs. 1–4):

Total no. of frames	No. of frames with *O. fragilis*	Total no. of *O. fragilis*	Mean no. of *O. fragilis* per ¼ m.^{2} frame
110	99	3143	32

These photographs therefore show a mean density of over 120 *O. fragi* per m.^{2} in the area traversed by the present transect.

Apart from this crowded population of *O. fragilis* the epifauna in this ar was poor, and the only other animals shown in the 110 interpreted fram presenting 275 m.^{2}) were 30 *Ophiocomina nigra*, 5 *Ophiura texturata*, *larthasterias glacialis*, 1 *Asterias rubens*, 1 *Echinus esculentus*, 5 *Eupagurus leauxi*, 5 *Chlamys opercularis*, 7 burrowing anemones, 3 hydroids and lump of *Cellaria* sp. These figures suggest that very few other epifaunal ertebrates live in an area with a crowded population of brittle-stars. It was, wever, noticed that the brittle-stars tend to avoid the area immediately rounding a burrowing anemone; this is shown in Pl. I, fig. 4.

Area North-west of Eddystone, ¾ Mile, Depth 48 m.

18 July 1951, R.V. *Sabella* was allowed to drift from position Eddystone ring 147° T., 0·73 miles, to Eddystone bearing 136° T., 0·80 miles, while first film was taken. The ship then steamed back to the first position and second film was taken along the same transect.

he photographs on these two films show the greatest density of brittle-rs recorded in the present investigation:

Total no. of frames interpreted	No. of frames with *O. fragilis*	Total no. of *O. fragilis*	Mean no. of *O. fragilis* per ¼ m.^{2} frame
36	36	3053	85

his gives a mean density of 340 *O. fragilis* per m.^{2}. These 36 ¼ m.^{2} frames contained 22 *Ophiocomina nigra*, 1 *Marthasterias glacialis* and 1 *Echinus lentus*.

he photographs from this transect (Pl. I, figs. 5–7) show that the individual tle-stars, although much more numerous, were also considerably smaller n those photographed in the other two brittle-star areas (south of Looe and a of Station L4). The figure of 340 individuals per m.^{2} may be regarded as ninimum, since in most frames the brittle-stars were living on top of each ier, and the counts made could only reckon the top layer and those disks animals in the lower layer which were not obscured by the tangled arms of top layer.

Distribution of *Ophiocomina nigra*

e black brittle-star, *O. nigra*, is common in the Plymouth area, but never such large numbers as *Ophiothrix fragilis*, with which it is nearly always ociated in dredge and trawl hauls. The present series of photographs gives opportunity of determining the proportions in which it occurs in these xed populations. From the figures in Table II it appears that there is, on average, less than 1 *Ophiocomina nigra* to 100 *Ophiothrix fragilis*.

TABLE II

Date	Locality	*O. fragilis*	*O. nigra*	Percentage of *O. nigra* in total brittle-star population
24. xi. 50	S.W. of Rame Head	547	2	0·4
15. ii. 51	S.W. of Rame Head	436	0	0
24. iv. 51	S. of Looe Island	763	1	0·1
25. vii. 51	S. of Looe Island	3143	30	0·95
18. vii. 51	N.W. of Eddystone	3053	22	0·72

DISCUSSION

There is now no doubt that the dense population of *Ophiothrix fragilis* 5 miles south of Looe Island, is not a seasonal phenomenon and that it is o a more permanent nature. Photographs of these brittle-stars have been take on three different occasions in 1950–1, and from them it appears that thi population is at least 2 miles in length and ½ mile in breadth, and it may b considerably larger. The mean density of brittle-stars in this area, calculate from the large number of photographs taken on the three transects, is abou 97 per m.^{2}, or 392,000 per acre. The amount of food, in the form of animal an plant detritus, consumed by such a population must be very large, and furthe work is in progress to determine the food requirements of this type of detritus eating brittle-star.

In the *Ophiothrix* population living ¾ mile north-west of Eddystone th minimum figures are 340 per m.^{2} or about 1·3 million per acre; here th individual brittle-stars were somewhat smaller than those in the populatio south of Looe Island. These photographs were taken, by chance, in th middle of Allen's (1899) Ground X, coarse gravel and muddy sand, whic he speaks of as characterized 'by the very great abundance of *O. fragili* which occurs almost to the exclusion of every other species'. His catche were taken with a dredge so that he had no exact measure of abundanc but there is little doubt that his *Ophiothrix* ground sampled at the en of the nineteenth century is essentially the same as that photographed i 1951.

The permanent nature of these brittle-star populations suggests that the rely for their food supply more on a steady flow of suspended material tha on a settled bed of living or dead organic matter. It is possible that much this suspended matter is carried back and forwards over the *Ophiothrix* be by the tidal streams, and the position of the Looe and Rame beds at 5 an 4 miles respectively from the coast would favour this. The tidal streams in th neighbourhood of Eddystone are well known, and they would also tend gather suspended material and carry it over the massed brittle-stars with the food-collecting net of tangled arms.

SUMMARY

improved underwater photographic apparatus has been used to take
ther series of photographs of the sea bottom near Plymouth. The photo-
phs in the present series are each $\frac{1}{4}$ m.^{2} in area instead of 1 m.^{2}, and at this
le definition is much better.

Photographs of the bottom in the Rame Mud area showed ripple marks but
living epifaunal animals. In an area south of the Rame Mud, on a muddy
d and gravel bottom and in a similar area 6 miles south of Looe, dense
pulations of the brittle-star, *Ophiothrix fragilis* were photographed. In the
a south of Looe this type of population (density more than 100 individuals
m.^{2}) has been photographed on three different occasions in 1950–1. In
imilar but still denser *Ophiothrix* population found $\frac{3}{4}$ mile north-west of
dystone, there were about 340 individuals per m.^{2}, and this aggregation
s apparently of long standing, since exceptionally large dredge hauls of
hiothrix were taken there in the last decade of the nineteenth century. It is
ggested that the food supply for these populations, in the form of suspended
terial, is brought to them largely by the tidal streams and that the crowded
ds are in localities where this is likely to happen.

From the photographic data it appears that in these dense populations of
ttle-stars there is rather less than 1 *Ophiocomina nigra* to 100 *Ophiothrix*
gilis.

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VERS, H. G., 1951. Photography of the sea floor. *Journ. Mar. Biol. Assoc.*, Vol. 30, pp. 101–11.

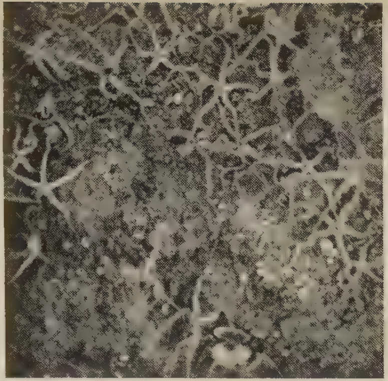
EXPLANATION OF PLATE I

Underwater photographs, each covering $\frac{1}{4}$ m.^{2} of the sea bottom.

Figs. 1–4. Showing samples from a dense population of *Ophiothrix fragilis*, 5 miles south o Looe Island. Density of over 100 brittle-stars per m.^{2}. In fig. 4 a burrowing anemone (where lines *a* and *b* cross) appears in a clearing among the brittle-stars. Depth 55 m.

Fig. 5–7. Showing samples from a dense population of *O. fragilis*, $\frac{3}{4}$ mile north-west o Eddystone. Density of about 340 brittle-stars per m.^{2}; individual brittle-stars smalle sized than in figs. 1–4. In fig. 6 the bottom is rock and a single *Echinus esculentus* is attache to a vertical face. In fig. 7 the bottom is shell gravel. Depth 48 m.

Fig. 8. Barren ground of muddy sand and gravel with shell fragments but no living epifauna invertebrates. One mile west of Station L4. Depth 54 m.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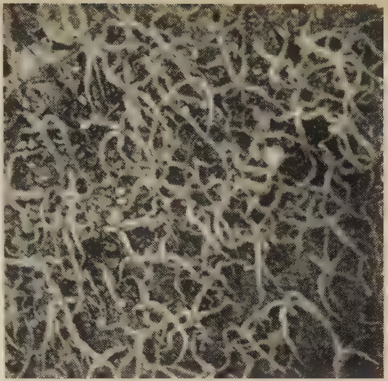
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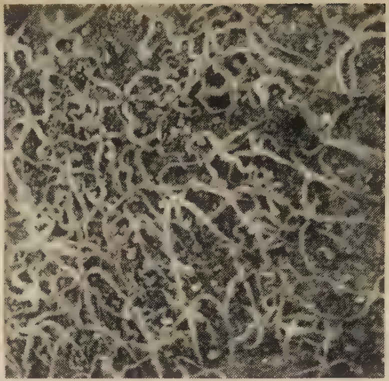
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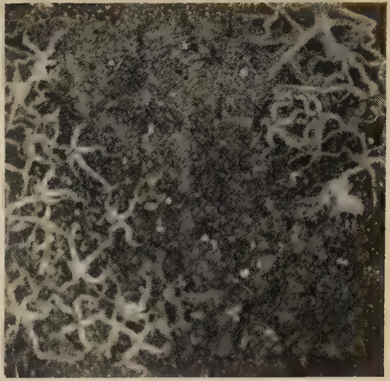
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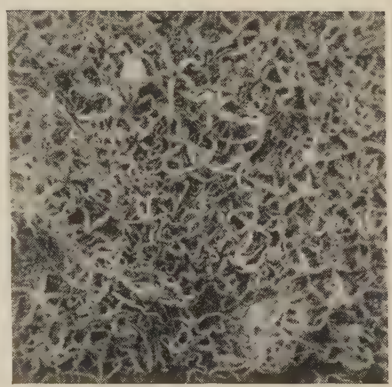
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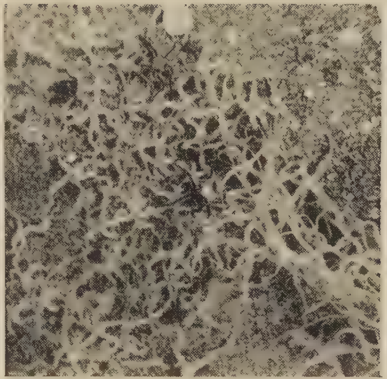
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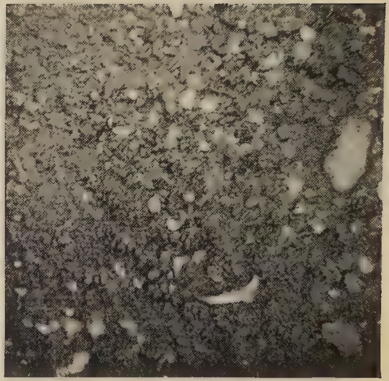
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OBSERVATIONS IN THE SEA ON THE REACTION TO ULTRA-VIOLET LIGHT OF CERTAIN SOUND SCATTERERS

By R. E. Craig and I. G. Baxter

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(Text-figs. 1 and 2)

he use of lanterns to attract herring is reported for British waters as early the fifteenth century (Jenkins, 1927). No such methods are in use here -day, although in Australia positive results have been reported in the case pilchard, and in Japan and the Far East the use of light lures has become aditional (Maeda, 1951).

The physiological importance of the ultra-violet component of natural ght prompts us to consider it equally with visible light as a possible stimulus. he human eye has become adapted to the red-violet range, centred on llow, which is the strongest component of the sun's spectrum at the surface. sea water there is differential absorption so that the centre of maximum tensity is displaced somewhat towards shorter wave-lengths, the precise fect depending upon depth and the nature of the sea water. We should not, erefore, be surprised to find marine creatures sensitive to a range including portion of the ultra-violet spectrum.

An earlier experiment, using visible light, has already been reported arrish & Craig, 1951). This showed that some organisms producing echo-aces are affected by light, and that certain deductions are thus possible bout the nature of these organisms.

Ultra-violet light has been known to attract trout and pike ('Reflector', 49). Experiments on herring conducted off the Belgian coast by the etherlands research vessel, however, did not lead to definite results (de oer, 1950). A number of experiments have been carried out on the East oast during the past summer, by Mr Parrish and the present authors. In ese an ultra-violet lamp was lowered where echo-traces were found. The aces were of various ill-defined forms, but included a typical shallow attering layer. No reaction was observed in any case.

We report here two experiments in which positive results have been btained using the ultra-violet light.

The source was a 125 W. 'black' ultra-violet lamp housed in a vertical lass cylinder with cast-iron ends. The horizontal intensity is therefore much greater than the vertical, and the upward and downward components are not dissimilar. When this lamp is lowered at night to a few fathoms depth in the summer sea, it produces at first no visible illumination. After about 5 min. a violet glow is visible in the surrounding water, and this intensifies until it can be seen to extend 3 to 4 m. from the lamp, the shadows of the lamp bolts cutting clear lanes through the luminescence. We take this to indicate that numerous fluorescent organisms are attracted by the light, though these have not yet been identified. The gathering of such a mass of small organisms may obviously affect the behaviour of their predators, and in work such as this it is necessary to distinguish between such secondary effects which must take some little time to develop, and immediate reactions to switching *on* of a light, which must presumably imply direct visual stimulus. The switching *off* of the lamp, however, is a visible event even to the human eye since the distinct luminescent glow vanishes abruptly.

Traces in Figs. 1 and 2 were obtained on board the R.V. *Clupea*, using a Kelvin and Hughes recording echo-sounder, type MS 22.

Recording I (see Fig. 1). Position: 7 miles S.E. × S. from Stornoway in 100 m. of water. Recording begins 20.10 hr. and ends 22.00 hr. 25 September 1951. Sky overcast, no moon. The ship is lying to drift nets.

A confused trace with a peculiar 'saw edge' appearance to its upper position extends from 20 to 60 m. depth, and the lamp is lowered into the centre of this to a depth of 35 m., appearing on the recording as a distinct horizontal line. Some avoiding action is evident and then the trace increases in density around the lamp. When this is switched on separation takes place into two distinct traces, the upper lying between 8 and 14 m. above the lamp, and the lower between 6 and 14 m. below it. The upper trace consists of crescents each of which may be attributed either to a single subject or to a small distinct group, the lower is of homogeneous appearance and may represent a more uniform distribution of scatterers (though it must be remembered that greater depth in itself gives a more uniform appearance to any distribution of this kind, owing to the larger cross-section area of the supersonic beam). We consider this lower trace to be similar in cause to the scattering layers described by Burd & Lee (1951) and Parrish & Craig (1951).

Half an hour later, after sampling has been carried out with the Hensen net to 38 m., the lamp is raised to 20 m., whereupon the lower trace rises, maintaining a constant depth below the light, and the upper trace descends to merge with the lower, forming a dense trace in which the components are not readily separable. Ten minutes later the light, still switched on, is lowered rapidly to 50 m., driving before it some fast-moving organisms, and then to 80 m. at which depth it is out of range of the echo-sounder and the trace returns above it. In this connexion it must be remembered that the space close to the light, which is devoid of scatterers, is probably in the form

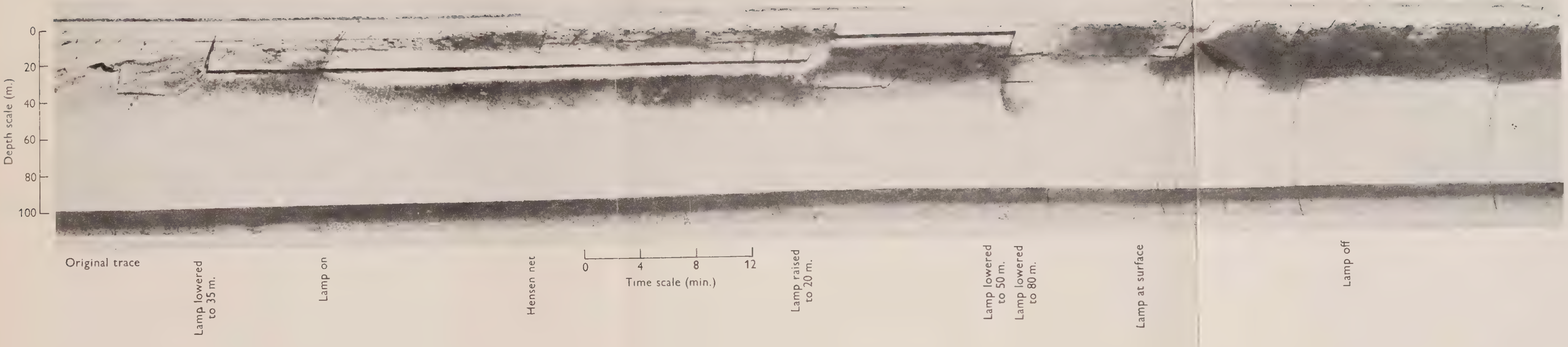

Fig. 1.

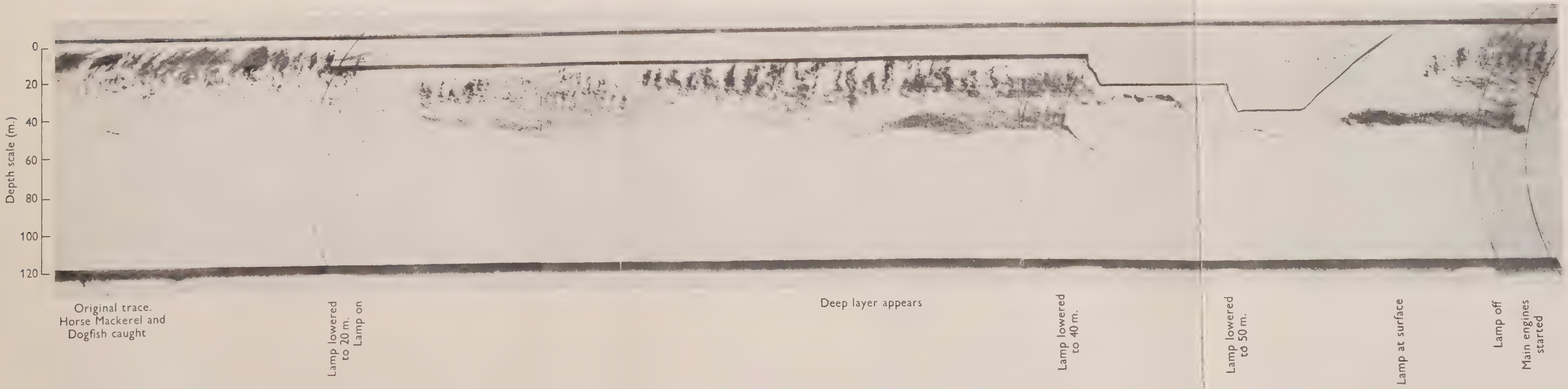

Fig. 2.

(*face* p. 224)

a sphere, and that to the side we have presumably an undifferentiated
er of scatterers. The reappearance of the shallow trace may therefore be
plained as a horizontal concentration of organisms, and does not necessarily
ply the return of those forced down by the light. Finally the lamp is raised
wly to the surface and remains there for a while switched on. The trace
ppears as a very dense band between 10 and 40 m., and remains thus even
er the lamp is switched off. An attempt to affect this trace by directing the
am of an Aldis signal-lamp downwards through the water produced no
tain reaction. The rate of apparent vertical movement of the lower
mogeneous) trace may be stated with reasonable accuracy as: descent,
n./min.; ascent, 5 m./min. The rate of movement of the upper (hetero-
neous) trace cannot be accurately assessed, but it is clearly much greater
n this.

The Hensen sample revealed that copepods were abundant, euphausids,
capod larvae and *Sagitta* common, and others present in small numbers
luded pteropods and hyperiids. This is not significantly different from
ner plankton hauls in the Minch at this season.

The drift net catch consisted of 39 herring, 24 common mackerel, 1 horse
ckerel, 5 young dogfish and 1 whiting, and the trace originally observed
s within reach of these nets, so we can feel certain that these are not caused
abundant adult herring. Fishing with 'dandy' lines and with baited
nd-lines throughout this experiment produced no result.

Recording II (see Fig. 2). Position: 6 miles S.E. from Stornoway in 120 m.
water. Recording begins 20.50 hr. and ends at 22.30 hr. 1 October 1951.
ear sky, no moon. The ship is again lying to drift nets.

A peculiar jagged trace is observed superimposed on one of spotty
pearance, each of these extending roughly from 8 to 30 m. depth. A few ill-
fined marks appear below this. The 'jags' are caused by objects rising very
iformly at about 14 m./min. and these are not seen descending. They could
caused either by gas bubbles, or by fish swimming up under the ship and
en away horizontally. The former would appear to be a more probable
planation[1] and presumably indicates the presence of physostomatous fish.
tches of horse mackerel (*Caranx trachurus*) and dogfish (*Squalus acanthias*)
re obtained at this position using a 'dandy line' which carries a series of
re hooks.

When the lamp is lowered into the centre of the trace some avoiding
ion is apparent, and when it is switched on, the trace disappears for about
min. and then begins to re-form under the light, the spotty component
urning first, followed after a lapse of another 10 min. by heavier markings

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[1] The literature on the rising velocity of bubbles has been summarized by Worster (1948),
l reference to his figures show that for bubbles between 0·04 and 1·4 cm. diameter, the
e of rise is roughly independent of size, and is of the order of 15 m./min. This would
ear to confirm our conclusion.

which may be caused by the same fish as the original jagged trace but no longer rising. Some 15 min. later two components can still be distinguished the lower homogeneous one maintaining a depth of about 20 m. below the lamp, whilst the top of the upper is at exactly the depth of the lamp. This might be explained as a reorganization within the layer, but the appearance is more suggestive of the arrival on the scene of fresh recruits.

By 21.55 hr. the lamp has been at the 20 m. mark for 50 min., and it is then lowered to 40 m. and subsequently to 50 m. *In a period of the order of* 1 *sec from the first movement* of the lamp, the topmost of the organisms producing the lower trace either descend 12 m. or remove themselves horizontally from the scene of action. The lower organisms appear to descend more sedately, remaining at a constant depth below the lamp, and then disappear The upper layer maintains its distance below the lamp, and also fades out.

The lamp is then hauled slowly to surface and remains there switched on for a time. The lower trace reappears at 50 m. depth and remains there, and the spotty trace re-forms. The immediate effect on the lower trace of the sound of starting up the main engine is of interest.

The drift net catch consisted of 31 herring, 44 horse mackerel, 24 common mackerel and 77 dogfish, and we can therefore exclude abundant adult herring as the cause of the upper water trace, although *not* as a possible cause of the lower one which is below the reach of the nets. In fact, the speed of reaction to stimulus of the latter is highly suggestive of herring or other active fish.

These experiments are not by themselves conclusive, and it is particularly unfortunate that the underwater camera was not available on this cruise When we lay the evidence alongside the work of many other investigators however, we can suggest associating the traces as follows:

(1)	Recording I	Upper trace	Fish, unidentified
(2)	Recording I	Lower trace	Plankton organisms, unidentified
(3)	Recording II	Spotty trace	Dogfish
(4)	Recording II	Jagged trace	Horse mackerel
(5)	Recording II	Deep trace	Probably herring, though whiting or other adult fish are a possible cause

It will be seen that results such as these are of intrinsic biological interest in that reactions to light can be measured using the echo-sounder. They also give hope of deriving ultimately a set of tests which would be of commercial value by giving greater certainty in the identification of the causes of echo traces. For both these reasons, experiments of this type will continue during the present year.

SUMMARY

wo experiments on the reaction to ultra-violet light of certain sound catterers in the area are described.

The source was a 125 W. ultra-violet lamp.

The scatterers were detected using a Kelvin and Hughes recording echo-ounder, type 22.

The traces on both occasions were of the confused type caused by a number f different organisms, apparently lying between 20 and 60 m. and 8 and 0 m. respectively.

The responses of the different organisms to the ultra-violet light as shown y the components of the traces are described and their speeds of reaction alculated.

The types of organisms causing the traces are suggested.

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ITAMIN A AND CAROTENOIDS IN CERTAIN INVERTEBRATES. I. MARINE CRUSTACEA

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(Text-figs. 1–6)

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INTRODUCTION

has for long been accepted that carotene is converted in the liver to tamin A, but recent work has established that, in several mammalian species any rate, the conversion takes place in the small intestine (see, for example, e recent review by Kon & Thompson, 1951).

During the study of the conversion of β-carotene to vitamin A in the intes- ne of rats and pigs (Thompson, Ganguly & Kon, 1947, 1949), Prof. B. C. P. nsen of Amsterdam drew our attention to the work of Wagner (1939), who aimed to have observed the phenomenon in the intestine of blue and fin hales at the Lopra Whaling Station in the Faeroes and stated that they rived the β-carotene from krill ('Gattung der *Euphausia superba* Dana'). *superba* Dana is exclusively antarctic, so that the krill which Wagner amined must have been composed of other Crustacea, of which the most ely species in the krill of Faeroese waters is *Meganyctiphanes norvegica* I. Sars). Wagner claimed that in his krill β-carotene was present to the tent of 14·5 mg./kg. This was suprising, since astaxanthin is the character- ic carotenoid pigment of Crustacea and the presence of such relatively ormous quantities of β-carotene did not seem likely.

Thompson *et al.* (1949) obtained, therefore, through the courtesy of r Robinson of the whaling factory ship *Balaena*, samples of food from the omachs and intestines, and portions of the intestinal wall, of two antarctic fin whales. The krill from these whales was identified as *Euphausia super* Dana. It contained large quantities of astaxanthin but only small traces β-carotene. Preformed vitamin A, identified, after chromatography, by t antimony-trichloride test, absorption at 328 mμ, behaviour on mixed chr matography with pure vitamin A and liver-storage tests with rats deplete of vitamin A, was present, however, in appreciable amounts, in one of t whales to the extent of about 6 i.u./g. stomach contents, with smaller co centrations farther along the intestine. Astaxanthin occurred in the intestin contents in increasing concentration along the gut; no carotene was detecte (Kon & Thompson, 1949*a*).

In northern waters, the main food supply of blue and fin whales is derive from the euphausiids, *Meganyctiphanes norvegica* and *Thysanoessa inerm* Krøyer (Einarsson, 1945). With the kind help of the workers at the Millpo Marine Station, Kon & Thompson (1949*b*) got from Loch Fyne specime of *Meganyctiphanes norvegica* and of *Thysanoessa raschii* (M. Sars), close related to *T. inermis*, and found much larger quantities of vitamin A than i other Eucarida caught there simultaneously.

The occurrence of vitamin A or its precursors in marine invertebrates h been studied by others since Hjort (1922), by biological tests with rats, detecte vitamin A activity in extracts from some marine plants and animals, includin *Crangon* sp. and *Pandalus borealis*. The evidence, however, is fragmenta and often conflicting; in several instances the total vitamin A activit was measured biologically, leaving undecided the presence of preforme vitamin A.

Drummond & Hilditch (1930) reported that copepods, *Nephrops norvegicu* and *Meganyctiphanes norvegica* contained little or no vitamin A.

Drummond & Gunther (1930, 1934), studying fatty constituents of mari plankton, looked for vitamin A but concluded that 'vitamin A as such apparently absent from both phytoplankton and zooplankton'. The stat ment was based for phytoplankton on an examination of oils isolated fro the diatoms *Chaetoceras* spp. and *Lauderia borealis*, and for zooplankton o a similar study of mixed copepods and *Calanus finmarchicus* (Collin, Dru mond, Hilditch & Gunther, 1934). In *Calanus* caught at the end of May o the north coast of Norway, Lederer (1938) found no trace of vitamin Drummond & MacWalter (1935) examined oil from antarctic krill (whic consisted mainly of *Euphausia* sp.), but did not look specifically for vitamin Extracts of the non-saponifiable portion contained a pigment which ga a slightly blue-green colour with antimony trichloride. They pointed ou with reference to earlier work based on biological tests, that certain isomer forms of carotene, among the naturally occurring lipochromes, are converte into vitamin A by the rat. Gillam, El Ridi & Wimpenny (1939) studied chemical and physical methods the seasonal variation of vitamin A in gro plankton samples from the North Sea; the highest content of vitamin incided with the first phytoplankton maximum and the zooplankton eeding period, diminishing later in the year.

Pugsley (1941) found vitamin A to the extent of 600 i.u./g. in oil extracted m the viscera of tinned crabs (*Cancer magister*); the oil constituted 6 % of e weight of these tissues.

The retina of the squid, *Loligo pealii*, contains 1–2 μg. of vitamin A_1 Vald, 1941) and about three times this amount of retinene_1, and no trace of ese or other carotenoids was found in other squid tissues, but the eyes of the abs *Uca pugnax* and *Carcinus maenas* and those of the lobster (unspecified) ntain high concentrations of vitamin A_1, but no retinene. In the squid tamin A remains constant in all conditions of light and darkness and does t, therefore, appear to participate directly in the visual processes. Wald 943) also found vitamin A_1, retinene_1 and astaxanthin in the eyes of the esh-water crayfish, *Cambarus virilis*. Neilands (1947) studied the conrsion of carotene to vitamin A in the lobster, *Homarus americanus*, and und 36 i.u./g. in the hepatopancreas and 100 i.u./g. in the eyes on a carotenee diet, and 53 i.u./g. in the hepatopancreas and 183 i.u./g. in the eyes on diet supplemented with β-carotene.

Recent developments in the micro-analysis of vitamin A in the presence a large excess of carotenoids made it possible to undertake a more detailed d systematic study than those hitherto attempted. The present paper is tly concerned with work on krill outlined above and in subsequent studies blished only in abstract (Batham, Fisher, Henry, Kon & Thompson, 1951; sher, Kon & Thompson, 1951) and partly with work on other species and geographical, developmental and seasonal variations and anatomical tribution of vitamin A in those animals found to possess it.

Material and Methods of Collection

he material consisted of plankton collected in Loch Fyne, the Faeroeetland area and north-west of the coast of Norway; krill obtained from hales in arctic and antarctic waters, and littoral and benthic animals from och Fyne and the Essex coast.

Regular visits were made to the Marine Station, Millport, at monthly, or ghtly less frequent, intervals, and the principal species obtained were *eganyctiphanes norvegica* (M. Sars), *Thysanoessa raschii* (M. Sars), *Euchaeta rvegica* Boeck, *Calanus finmarchicus* (Gunnerus), *Crangon allmani* Kinahan, *ndalus bonnieri* Caullery and *Nephrops norvegicus* L. The Faeroe-Shetland ea was visited in the Scottish Home Department's Fisheries Research essel *Scotia* during November 1950, and *Meganyctiphanes norvegica* and *hysanoessa inermis* Krøyer were the most important animals brought back om these waters. Antarctic krill was kindly supplied from the W.F.S. *alaena*, and arctic specimens were taken from a fin whale caught 200 miles north-north-west of Bergen and also free-swimming from the same area in June 1950, by catcher *Hval* 2 from Blomvåg Hvalstasjon, Norway, an from a blue whale caught 40 miles north-west of St Kilda by a catcher from the whaling station of Scottish Whalers Limited, West Loch, Tarbert, Harris Littoral and benthic Crustacea were collected at Burnham-on-Crouch an other parts of the Essex coast and included *Carcinus maenas* (Pennant) *Eupagurus bernhardus* (L.) and *Gammarus marinus* Leach.

Initially specimens were placed as soon as possible after catching i a measured volume of absolute alcohol, after drying with filter paper. Aluminium containers were used for lightness, chemical inertness and opacity Alcohol is an excellent preservative for vitamin A and carotenoids, but, sinc it leaches them out, distribution in the various organs cannot be studied an only the total content can be measured. Later it was found more convenien and accurate to place specimens, immediately after catching, for a minute o so in boiling sea water. Boiling fixes vitamin A and carotenoids in thei original sites and anatomical separation is later possible. As shown below, th method also allows more accurate weighing of the specimens. The specimen thus treated were transported in aluminium containers without preservativ and kept as cool as possible. The greatest attention was always paid t protection from strong light, in order to reduce photochemical effects.

Chemical and Physical Tests

Methods

Weighing of Specimens

The weight of the alcohol-preserved specimens was determined by weighin the containers full and again empty. The volume of absolute alcohol bein known, the weight of the animals or tissues could be calculated, but indire weighing was not accurate enough with small animals or parts of animal

Table I. Recovery of Oil, Vitamin A and Carotenoids from Specimen of *Meganyctiphanes norvegica* preserved by Alcohol (A or by brief Boiling in Sea-Water (B)

Preserved by	Length (cm.)	No. of specimens	Oil (mg./specimen)	Vitamin A (i.u./specimen)	Astaxanthin (μg./specimen
A	<3	238	3	1·2	4·5
B	<3	93	3	1·3	4·8
A	3–4	95	18	4·2	16
B	3–4	60	20	5·4	23
A	>4	44	44	27	44
B	>4	20	39	26	44

Comparison of alcohol-preserving with boiling showed equal recovery o vitamin A and carotenoids by both methods (Table I). As a further check th right eye of each of a group of 150 *Meganyctiphanes norvegica*, all of avera length greater than 40 mm., was removed and preserved in alcohol. Th

mals were then boiled and the left eyes were removed. The two lots of
s were analysed separately with the following results:

	Vitamin A (i.u./eye)	Carotenoids (μg./eye)
Eyes preserved in alcohol	10·9	4·7
Eyes boiled	11·7	5·0

e water in which the specimens had been boiled was examined and no
ce of vitamin A or carotenoids was found in it.

To determine the effect of boiling on the weight of the animals, ten
norvegica were weighed alive after removal of surplus moisture, and ten
er boiling; they were then dried at 105° C. for 20 hr. The effect of boiling
negligible, since the live lost 69 % and the boiled 72 % of their weight on
ring.

After any necessary dissection had been completed in red light, boiled
cimens or parts of them were weighed and preserved in alcohol.

traction of Lipids, Vitamin A and Carotenoids and Measurement of Total rotenoids

This was done as described by Thompson *et al.* (1949). Specimens were
cerated in a Waring Blendor jar, proportions taken for each homogeniza-
n being about 20 g. of tissue, 60 ml. absolute alcohol and 200 ml. light
oleum (b.p. 40–60° C.). Before maceration, nitrogen was bubbled through
mixture and into the mouth of the jar which was then closed with a lid.
e mixture was homogenized for about 2 min., transferred to a separating
nel and the bottom layer was run off and re-extracted with a further
ml. of light petroleum. The two extracts were combined, the volume
ermined and the total carotenoids measured in the photoelectric spectro-
otometer of Thompson (1949) at 451 mμ, that is, at the absorption maxi-
m for β-carotene. For animals whose only carotenoid was astaxanthin this
ding was used as a measure of the pigment. Values obtained by this means
re probably some 10 % lower than those based on the measurement of
axanthin at its absorption maximum and referred to the extinction for the
re substance.

The solvent was evaporated and the oil weighed and dissolved in *n*-hexane.

paration of Vitamin A from β-Carotene and other Carotenoids

The chromatographic method of Thompson *et al.* (1949), employing alu-
na columns, was used. The separation and measurement of vitamin A,
al carotenoids and β-carotene is shown schematically in Fig. 1. Two
ernatives could be followed: (*a*) direct saponification of the extract in
hexane and subsequent chromatography on aluminium oxide; (*b*) direct
romatography of the extract which separated vitamin A ester and alcohol.
(*b*), the two fractions were saponified and rechromatographed to obtain

a further purification from carotenoids of the vitamin A, now in the alcoho form. Method (*a*) was used for routine work where a separation of the tw

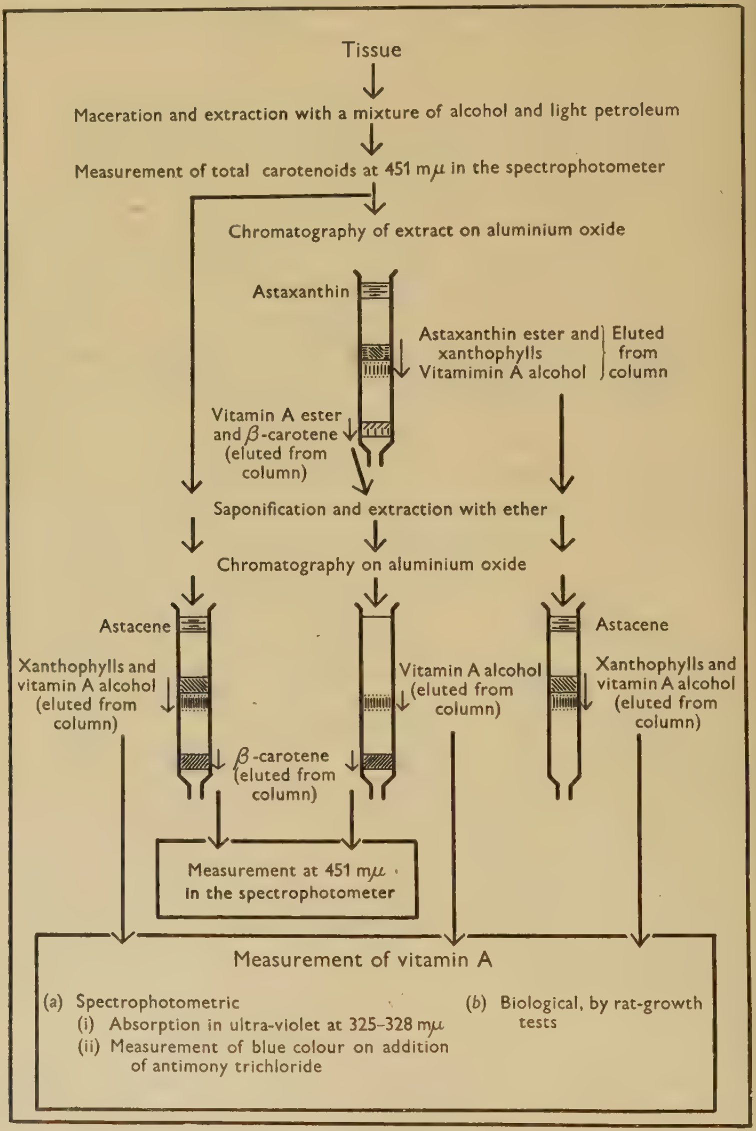
Tissue
Maceration and extraction with a mixture of alcohol and light petroleum
Measurement of total carotenoids at 451 mμ in the spectrophotometer
Chromatography of extract on aluminium oxide
Astaxanthin
Astaxanthin ester and xanthophylls Vitamimin A alcohol
Eluted from column
Vitamin A ester and β-carotene (eluted from column)
Saponification and extraction with ether
Chromatography on aluminium oxide
Astacene
Xanthophylls and vitamin A alcohol (eluted from column)
β-carotene (eluted from column)
Vitamin A alcohol (eluted from column)
Astacene
Xanthophylls and vitamin A alcohol (eluted from column)
Measurement at 451 mμ in the spectrophotometer
Measurement of vitamin A
(a) Spectrophotometric
(i) Absorption in ultra-violet at 325–328 mμ
(ii) Measurement of blue colour on addition of antimony trichloride
(b) Biological, by rat-growth tests

Fig. 1. Outline of methods of separation and measurement of vitamin A, astaxanthin and β-carotene in marine animals.

forms was not required and the pigments were such that they did not co taminate the vitamin A fraction. β-Carotene, if present, was separated either method and collected at the stages indicated in the figure.

astaxanthin and xanthophyllic pigments, if present, were not, as a routine cedure, measured after chromatography. It will be noticed from the figure we were unable to separate xanthophylls from vitamin A alcohol. Where se pigments were present in relatively large quantities they vitiated the ermination of vitamin A in this form. The difficulty did not arise with hausiids and was probably of little consequence with the other Crustacea. The fractions containing vitamin A were taken up in 1·5 ml. chloroform that containing β-carotene in 5 ml. *n*-hexane. Vitamin A was measured the antimony-trichloride reaction, and β-carotene determined on the otoelectric spectrophotometer as described by Thompson (1949) and ompson *et al.* (1949). Absorption curves for vitamin A in ultra-violet and β-carotene and astaxanthin in ultra-violet and visible light were obtained the Beckman quartz photoelectric spectrophotometer.

For euphausiids, the following procedure was adopted for the identification astaxanthin and its ester. The absorption curve of the total extract in hexane was determined. The pigment was further identified by its behaviour chromatography (Goodwin & Srisukh, 1949) and its low solubility in diethyl er after saponification.

Results

Form of Vitamin A in the Crustacea

s stated above (p. 234) vitamin A ester and alcohol were not always arated in routine tests, and for a number of species only values for total amin A are at present available. Our observations on Euphausiacea and ain Decapoda show that in them vitamin A was present mainly as the er. Details are given in the sections dealing with the relevant species.

BLE II. Vitamin A and Carotenoids per gram in Gut Contents of Antarctic Fin Whales (Nos. 1 and 2) and of Blue Whale (No. 3)

	No. 1		No. 2		No. 3*	
	Vitamin A (i.u.)	Carotenoids (μg.)	Vitamin A (i.u.)	Carotenoids (μg.)	Vitamin A (i.u.)	Carotenoids (μg.)
Stomach	6·0	41	2·5	38	2·0	22
Small intestine	0·5	2·2	3·8	49	—	—
Caecum	2·1	89	4·3	79	—	—
Rectum	1·1	85	1·6	103	—	—

No. 3, blue whale, stomach oil 2·1 %, vitamin A 91 i.u./g. oil and carotenoids 1·02 mg./g.

ll from Antarctic Whales

Contents of the alimentary canals of two fin whales and one blue whale e examined. They had been preserved by deep-freezing, and were ntified by Prof. C. H. O'Donoghue as being mainly *Euphausia superba* na. Results obtained are shown in Table II.

The lower value obtained for the stomach contents of fin whale 2 was possibly correlated with their greater fluidity. They were either in a more advanced stage of digestion or contained less krill in proportion to the digestive juices. Judging from the appearance of the material the former explanation was more likely. In the light of later work (see p. 239), it is also possible that the difference in concentration of vitamin A was due to differences in the developmental stage and size of the krill eaten by the two fin whales. Vitamin A was present mainly in the ester form.

So far no free swimming *E. superba* has been available to us for an examination of its contents of vitamin A and carotenoids.

Organs of Antarctic Whales

Segments of the alimentary canals of the two fin whales whose stomach contents were analysed were also examined, along with organs from three blue whales, and the results obtained are shown in Table III. The gradien of vitamin A decreased from the small intestine to the rectum, in agreemen with our findings on the absorption of vitamin A in other mammals (Thompson *et al.* 1949). It seems possible that the absorption of vitamin A is incomplete as it was still found in the caecal and rectal contents.

TABLE III. VITAMIN A CONTENT (I.U./G.) IN ANTARCTIC FIN AND BLUE WHALES

		Fin no. 1	Fin no. 2
Small intestine:	Mucosa	0·34*	0·64†
	Wall	0·21	0·29
Caecum		0·12	0·26
Rectum		0·19	0·21

* Washed. † Unwashed.

Blue whale No. 1 (83 ft. lactating), liver 6300; No. 2 (88 ft. lactating), liver 6120; No. (87 ft.), kidney 3·6.

In contradistinction to vitamin A the pigments did not appear to b absorbed, since they became steadily more concentrated in their passag along the alimentary canal (Table II).

Krill from Arctic Whales

The contents of the alimentary canals of two whales were examined. On of these was a fin whale shot 200 miles from the coast of Norway and th other was a blue whale shot 40 miles north-west of St Kilda. The results ar shown in Table IV. In both instances the krill was identified as *Meganycti phanes norvegica* (M. Sars).

The low figures for vitamin A and astaxanthin in the mouth contents o the blue whale were probably due to considerable leaching out by sea wate while the whale was being towed 80 miles back to the whaling station a Tarbert. The krill in the mouth was much paler in colour than that collecte m farther along the gut. In the stomach of the blue whale, the euphausiids undergone little digestion and individual animals were still distinguish- e. The presence of krill in the mouth and oesophagus indicates possible urgitation, which also may have taken place between the four parts of the mach, as happens when a whale is shot. Unfortunately, no information vailable to indicate from which part of the stomach the contents were taken any of the whales, either arctic or antarctic, examined.

Table IV. Oil per cent, Vitamin A and Carotenoids per gram in the Gut Contents of Arctic Whales

	Oil (%)	Vitamin A (i.u./g.)	Vitamin A (i.u./g. oil)	Astaxanthin (μg./g.)	Astaxanthin (mg./g. oil)	β-Carotene
		Fin whale, 11. vi. 50, off Norway				
mach	0·8	1·5	188	22	2·81	Trace
ll intestine	0·1	2·5	2380	5·0	4·80	Trace
		Blue whale, 22. viii. 50, 40 miles north-west of St Kilda				
uth	0·7	0·5	87	1·6	0·24	None
ophagus	0·1	3·1	325	46	4·79	None
mach	0·1	5·0	392	48	3·78	Trace

Table V. Oil per cent, Vitamin A and Carotenoids per gram in Free-swimming *Meganyctiphanes norvegica* (M. Sars)

Locality	Date (1950)	Oil (%)	Vitamin A (i.u./g.)	Vitamin A (i.u./g. oil)	Astaxanthin (μg./g.)	β-Carotene
Loch Fyne	18. viii*	2·2	15	680	42	Faint trace
Loch Fyne	4. i	3·1	26	835	70	Trace
North Sea (63° 20′ N., 0° 30′ E.)	30. vi	5·3	15	288	46	None
Loch Fyne	17. viii	6·7	40	596	112	Trace
Sandy Bank, Faeroes (61° 54′ N., 5° 45′ W.)	6. xi	5·6	19	343	57	None

* 1949.

ee-swimming Arctic Krill

Meganyctiphanes norvegica. The most important species of euphausiid in tic krill, forming the food of baleen whales, is *Meganyctiphanes norvegica*, d, in order to obtain results comparable with those from animals taken m whales, free-swimming animals from different areas of the sea were amined. The work of Macdonald (1927) on this species in Loch Fyne licated that this sea-loch was a good and convenient source of supply. her samples of arctic krill were got from the Norwegian whaling area and m the Sandy Bank area, near the Faeroes. Table V shows the results of alyses of these specimens. Of the vitamin A, 94 % was in the ester form d was chromatographically homogeneous before saponification with pure amin A acetate and after saponification with vitamin A alcohol prepared m the acetate by saponification. The chromatographic homogeneity was served by fluorescence under ultra-violet light.

The Loch Fyne specimens were caught at depths of 120–140 m., about 20 m. from the bottom in a 1 m. stramin net. Those from the Norwegian whaling ground were taken from the surface of the sea in daylight. The haul in the Sandy Bank waters was made with a 1 m. coarse silk net at 11 p.m. at a depth of 20 m., where the sounding was 274 m.

The much higher concentration of vitamin A in the free-swimming animals than that in those from the whales is very striking, but we noted that the average overall length of specimens taken from the blue whale at Tarbert was only about 30 mm., whereas those caught in Loch Fyne were over 35 mm. in length. It was necessary, therefore, to investigate any possible relationship between size and vitamin A concentration. Conditions of sorting specimens on the ship where large numbers had to be dealt with as quickly as possible rendered the best means of size-grouping, i.e. weighing, impossible of application, and so overall length was taken as a criterion. In the initial experiments, three size-groups of specimens taken in Loch Fyne from hauls by the M.V. *Calanus* were examined, those animals less than 30 mm. long, those between 30 and 40 mm., and those over 40 mm. Each group was weighed in order to determine the average weight of individual members The results of these experiments are shown in Table VI.

TABLE VI. RELATIONSHIP BETWEEN OIL PER CENT, VITAMIN A AND CAROTENOIDS PER GRAM AND LENGTH AND WEIGHT IN *MEGANYCTIPHANES NORVEGICA* (M. SARS)

(β-Carotene absent)

Date	Length overall (cm.)	No. of specimens	Mean wt. (mg.)	Oil (%)	Vitamin A		Astaxanthin (µg./g.)
					(i.u./g.)	(i.u./g. oil)	
4. x. 50	<3	76	68	1·7	9·6	555	78
4. x. 50	3–4	131	320	10·1	13	131	55
4. x. 50	>4	53	500	8·6	34	397	60
21. xi. 50	<3	93	85	3·2	15	466	56
21. xi. 50	3–4	60	380	6·0	16	263	68
21. xi. 50	>4	20	580	6·7	44	660	75
10. i. 51	<3	3424	90	2·9	21	717	66
10. i. 51	3–4	1072	330	4·9	24	475	63
10. i. 51	>4	140	570	6·5	43	666	83

The concentration of vitamin A, even in the smallest of the size-groups, was still much higher than the highest obtained for *Meganyctiphanes* from the whale, even though the average size of these was greater.

The noteworthy feature of the table is the marked increase in the vitamin A content with size. At each examination the concentration was about the same in the two smaller groups but was more than doubled in the largest. The content of astaxanthin also increased, but the increase in concentration in the largest groups was much less marked than for vitamin A. β-Carotene was not present in sufficient quantity to be detected.

To determine more precisely the relationship between vitamin A content size, grouping of sizes was carried out in greater detail as a result of ctice and experience. On 14–15 February 1951, a series was obtained with ze interval of 2 mm. Macdonald's (1927) method of measuring the length n the tip of the rostrum to the base of the telson was also adopted at this ge, in preference to using the overall length as previously. These groups re weighed and analysed as before, and Table VII shows the results ained. The vitamin A content per specimen increased at first gradually, but increase became disproportionately great in the larger size-groups, and the centration, which was fairly constant in the smaller animals, showed an ward trend in the last four groups. Astaxanthin increased steadily in tent with the size of the animal without any clear-cut change in the centration.

LE VII. RELATIONSHIP BETWEEN OIL PER CENT, VITAMIN A AND CAROTENOIDS PER GRAM AND LENGTH AND WEIGHT IN *MEGANYCTIPHANES NORVEGICA* (M. SARS), TAKEN ON 14–15 FEBRUARY 1951, IN LOCH FYNE

(β-Carotene absent)

No. of specimens	Length* (mm.)	Mean wt. (mg.)	Oil (%)	Vitamin A (i.u./g.)	Vitamin A (i.u./g. oil)	Astaxanthin (μg./g.)
12	17	45	4·9	22	455	88
43	19	70	2·2	17	759	60
47	21	94	1·8	13	700	57
32	23	115	1·7	20	1171	51
25	25	155	2·4	21	858	54
49	27	209	2·0	21	1035	53
116	29	265	3·1	16	529	51
207	31	312	3·4	18	524	54
123	33	394	3·6	22	603	51
134	35	469	4·6	32	704	72
102	37	513	5·6	50	895	75
37	39	636	5·5	62	1120	76
8	41	738	5·3	58	1089	69

* Length measured from tip of rostrum to base of telson.

Fig. 2 presents graphically the relationship between vitamin A and astaxan- n content and weight of specimen. In Fig. 3 the concentrations of vitamin A astaxanthin have been plotted against size. The two substances measured w almost identical fluctuations.

Thysanoessa raschii. Next in importance to *Meganyctiphanes norvegica* as od animal for whales in arctic krill is *Thysanoessa inermis* Krøyer. Because closely related species *T. raschii*, which Einarsson (1945) regards as ord dweller, is available and obtained in large numbers in the same hauls *Meganyctiphanes norvegica* in Loch Fyne, vitamin A was also studied in s species of euphausiid. Presence of the vitamin in high concentrations is wn in Table VIII. The ester form accounted for 95 % of the total.

The much higher concentrations of both vitamin A and astaxanthin in the January haul compared with the two August samples, which agree well between themselves, indicated a possible seasonal variation, but all these groups were random collections of different-sized animals, and experienc

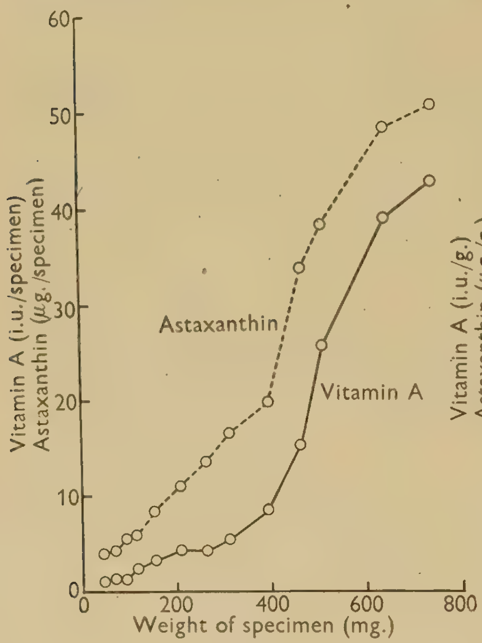

Fig. 2.

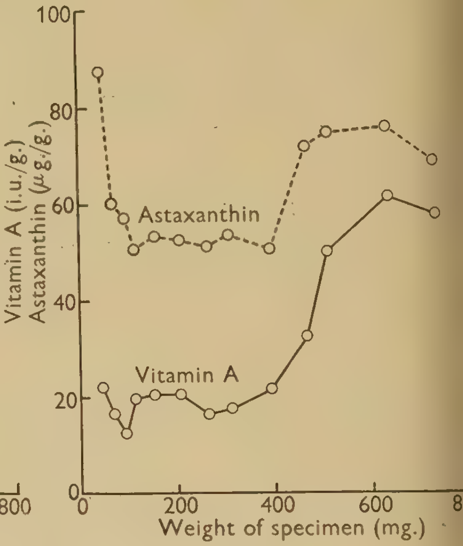

Fig. 3.

Fig. 2. Relationship between content of vitamin A and astaxanthin and size in *Meganycti-phanes norvegica* (M. Sars).

Fig. 3. Relationship between concentration of vitamin A and astaxanthin and size in *Mega-nyctiphanes norvegica* (M. Sars).

TABLE VIII. OIL PER CENT, VITAMIN A AND CAROTENOIDS PER GRAM IN *THYSANOESSA RASCHII* (M. SARS) FROM LOCH FYNE

(β-Carotene absent)

Date	Oil (%)	Vitamin A (i.u./g.)	(i.u./g. oil)	Astaxanthin (µg./g.)	β-Carotene
18. viii. 49	6·6	32	495	33	Faint trace
4. i. 50	5·6	76	1366	58	Trace
17. viii. 50	6·1	32	520	31	Trace

with *Meganyctiphanes* suggested that a similar relationship between vitamin content and size might occur in *Thysanoessa*. *T. raschii* was, therefor separated into two size-groups of less and more than 20 mm. long, and marked difference in vitamin A content was found, as is shown in Table I more detailed grouping of sizes was again done as with *Meganyctiphanes*, h results illustrated in Table X. With both vitamin A and astaxanthin content increased with size, but the concentrations fluctuated considerably. ese points are emphasized by the graphs in Figs. 4 and 5.

BLE IX. RELATIONSHIP BETWEEN OIL PER CENT, VITAMIN A AND CAROTENOIDS PER GRAM AND LENGTH AND WEIGHT IN *THYSANOESSA RASCHII* (M. SARS)

(β-Carotene absent)

Date	Length overall (cm.)	Mean wt. (mg.)	Oil (%)	Vitamin A (i.u./g.)	Vitamin A (i.u./g. oil)	Astaxanthin (μg./g.)
3. x. 50	<2	27	7·2	60	833	43
3. x. 50	>2	52	10·7	83	781	40
21. xi. 50	<2	24	6·9	51	734	38
21. xi. 50	>2	45	12·0	86	714	62
10. i. 51	<2	33	3·0	50	1680	44
10. i. 51	>2	81	3·0	69	2293	46

BLE X. RELATIONSHIP BETWEEN OIL PER CENT, VITAMIN A AND CAROTENOIDS PER GRAM AND LENGTH AND WEIGHT IN *THYSANOESSA RASCHII* (M. SARS), TAKEN ON 14–15 FEBRUARY 1951, IN LOCH FYNE

(β-Carotene absent)

No. of specimens	Length* (mm.)	Mean wt. (mg.)	Oil (%)	Vitamin A (i.u./g.)	Vitamin A (i.u./g. oil)	Astaxanthin (μg./g.)
32	11	10	6·6	77	1170	105
309	13	18	3·0	84	2790	58
479	15	23	2·9	75	2580	41
109	17	23	4·0	91	2270	54
30	19	38	4·9	113	2300	83
29	21	55	5·9	100	1690	37

* Length measured from tip of rostrum to base of telson.

Thysanoessa inermis. In hauls taken with 1 m. coarse silk nets at depths of 20 and 100 m. in the Sandy Bank area (61° 54′ N., 5° 45′ W.) at 11 p.m., ovember 1950, specimens of *T. inermis* were present in the tow-nets along h those of *Meganyctiphanes norvegica* already mentioned. The specimens re small, with a maximum length of 15 mm. in a species which, according Einarsson (1945), reaches up to 32 mm. The analytical results are shown in ble XI.

Vitamin A was present in one group in concentrations comparable with se in *Thysanoessa raschii*. The most significant feature of these results, wever, was that the oil content increased markedly with the depth from ich the specimens were taken, irrespective of their size as shown by weight d, more interesting still, the vitamin A content and concentration also reased in a similar fashion. The content of astaxanthin, on the other hand, l not appear to be related to the depth. In *Meganyctiphanes norvegica*,

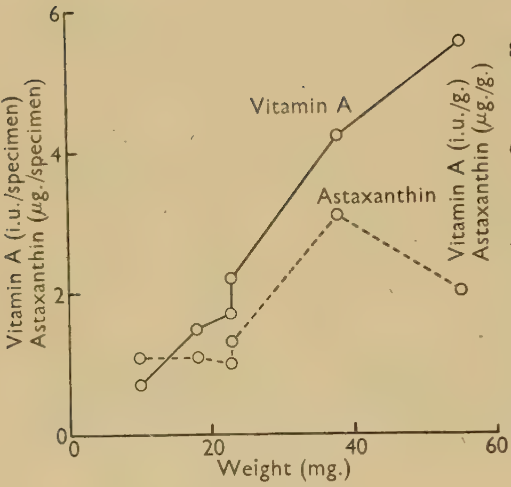

Fig. 4.

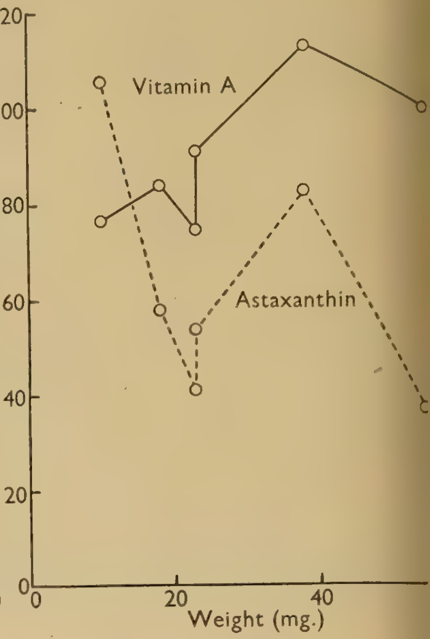

Fig. 5.

Fig. 4. Relationship between content of vitamin A and astaxanthin and size in *Thysanoessa raschii* (M. Sars).

Fig. 5. Relationship between concentration of vitamin A and astaxanthin and size in *Thysanoessa raschii* (M. Sars).

TABLE XI. OIL PER CENT, VITAMIN A AND CAROTENOIDS PER GRAM IN *THYSANOESSA INERMIS* KRØYER AND *MEGANYCTIPHANES NORVEGICA* (M. SARS) IN RELATION TO DEPTH OF HAUL

(β-Carotene absent)

Depth (m.)	No. of specimens	Mean wt. (mg.)	Oil (%)	Vitamin A (i.u./g.)	Vitamin A (i.u./g. oil)	Astaxanthin (µg./g.)
			Thyanoessa inermis			
0	374	8	5·2	5·7	110	73
20	253	11	8·3	12	148	66
100	124	7	12	55	474	86
			Meganyctiphanes norvegica			
0	91	80	3·9	16	410	65
20	200	100	5·6	19	343	57
100	225	100	4·1	16	381	55

en in the same hauls with *Thysanoessa inermis*, no relationship was evident ween content of vitamin A or oil and the depth. The results with *T. inermis* , so far, only for an isolated set, but indicate that further investigation of ossible relationship between depth of haul and vitamin A content in this cies may prove extremely interesting.

thic and Littoral Eucarida

So that comparisons with the Euphausiacea might be made, members of other order of the subclass Eucarida, the Decapoda, were studied. Some hese animals were taken from the sea-bottom in the areas of Loch Fyne m which the euphausiids were obtained, and others were from shallower ters of this and other coasts. Details of the species studied and the results analyses are given in Table XII.

The concentration of vitamin A was considerably less than anything found he Euphausiacea, although in some of the larger animals, such as *Portunus*, *dalus*, and *Nephrops*, the vitamin content of individuals was on a scale nparable with that found in single specimens of *Meganyctiphanes* and *ysanoessa*. *Crangon allmani*, *Pandalus bonnieri* and *Spirontocaris spinus* were taken from depths of 140 m. with an Agassiz trawl in waters from which ge numbers of *Meganyctiphanes* and *Thysanoessa* were caught in a 1 m. amin net attached to the warp of the trawl about 10 m. above it. The res for total carotenoids are given as such rather than for astaxanthin, e in some instances other pigments were present but were not isolated and ntified. The concentration of carotenoids was lower than that of astaxanthin the euphausiids, although larger species again showed high individual tents. β-Carotene was present in measurable quantities in oil extracted m *Eupagurus* and *Portunus*.

her Crustacea

Pelagic Crustacea taken in the same hauls with *Meganyctiphanes* and *ysanoessa* in Loch Fyne were the copepods, *Calanus finmarchicus* (Gun- rus) and *Euchaeta norvegica* Boeck. These were obtained on several occasions sufficient quantities for vitamin A analysis. Other Crustacea also examined re the copepod, *Euchaeta barbata* Brady, which was taken during a deep ique haul with a 1 m. silk net at midday on 7 November 1950, in the eroe-Shetland channel (61° 28′ N., 3° 42′ W.) with 1600 m. of warp out at ounding of 1200 m.; the cladoceran, *Evadne nordmanni* Lovén, taken off Norwegian coast, near Bergen; the littoral amphipod, *Gammarus marinus* ach; and the isopod, *Orchomenella nana* (Krøyer), of which many were nd feeding in the carapace of a dead crab. Table XIII shows the results tained from examinations of these species.

Lederer (1938) examined *Calanus* caught at the end of May off the north ast of Norway and found no vitamin A in it. The results given in the table

Table XII. Oil per cent, Vitamin A and Carotenoids per gram in some Decapods

Species	Date	Locality	Oil (%)	Vitamin A (i.u./specimen)	Vitamin A (i.u./g.)	Vitamin A (i.u./g oil)	Total carotenoids (µg./specimen)	Total carotenoids (µg./g.)	Total carotenoids (µg./g. oil)	β-Carotene (µg./g.)	β-Carotene (µg./g. oil)
Crangon allmani Kinahan	31. viii. 49	Loch Fyne	1·3	0·5	0·4	30	7·0	5·0	390	—	Trace
	21. xi. 50	Loch Fyne	1·6	0·3	0·4	23	2·7	3·8	238	—	None
	15. ii. 51	Loch Fyne	1·7	0·7	0·7	41	5·3	5·5	324	—	None
Crangon vulgaris L.	28. vii. 49	Bay of Holland, Stronsay, Orkneys	0·8	—	0·2	21	—	5·0	550	—	Trace
Eupagurus bernhardus (L.)	11. xii. 50	Burnham-on-Crouch	1·3	1·2	0·2	15	240	41	3170	4·8	369
Nephrops norvegicus L.	13. ii. 51	Loch Fyne	0·6	7·7	0·04	7·3	1850	11	1800	0·02	3·8
Pandalus bonnieri Caullery	30. viii. 49	Loch Fyne	2·3	8·9	2·1	89	101	24	1000	—	Faint trace
	15. ii. 51	Loch Fyne	1·6	7·2	1·5	94	225	46	2850	—	None
Portunus puber (L.)	4. i. 50	Loch Fyne	0·8	28	0·3	39	2150	24	3100	2·8	350
Spirontocaris spinus (Sowerby)	31. viii. 49	Loch Fyne	2·9	0·8	0·6	22	34	27	950	—	Trace
	15. ii. 51	Loch Fyne	1·4	1·0	1·1	79	24	28	1970	—	None

Table XIII. Oil per cent, Vitamin A and Carotenoids per gram in some Entomostraca and Peracarida

Vitamin A absent, save in *Euchaeta norvegica*, 4 January and 17 August, 0·3 i.u., with 2 and 12 i.u./g. oil.

Species	Date (1950)	Locality	Oil (%)	Total carotenoids (µg./g.)	Total carotenoids (µg./g. oil)	β-Carotene (µg./g.)	β-Carotene (µg./g. oil)
Calanus finmarchicus (Gunnerus)	4. i	Loch Fyne	1·6	7	420	—	Trace
	17. iv	Loch Fyne	2·0	18	970	0·1	7
	26. vi	Near Norwegian coast	6·2	57	918	—	None
	4. x	Loch Fyne	7·9	24	331	—	None
	7. xi	Faeroe-Shetland channel	9·5	12	122	—	None
Euchaeta norvegica Boeck	4. i	Loch Fyne	11	107	1000	1·3	12
	17. viii	Loch Fyne	2·7	21	770	—	None
	3. x	Loch Fyne	11	73	642	—	None
	5. iv*	Loch Fyne	5·1	19	367	—	None
Euchaeta barbata Brady	7. xi	Faeroe-Shetland channel	10	72	693	—	None
Evadne nordmanni Lovén	5. vi	Near Norwegian coast	0	0	0	—	None
Gammarus marinus Leach	18. ix	Essex coast	2·1	35	1660	—	Trace
Orchomenella nana (Krøyer)	10. x	Burnham-on-Crouch	1·4	13	922	—	Faint trace

vide a confirmation and extension of this observation, since the samples
e taken at other seasons and from different places, and show that vitamin A
ermanently absent from this species.

he first two extracts of *Euchaeta norvegica*, taken in January and August,
m hauls weighing 56 and 26 g. respectively, contained measurable quanti-
of vitamin A, since they gave, after chromatography, a good blue colour
n antimony trichloride, but subsequent samples, taken in October and
il and weighing 59 and 26 g., did not. In those specimens in which
min A was present, the concentration was, however, small (Table XIII).
e oil content and total carotenoid concentration also fluctuated to a con-
rable extent, and more seasonal data are necessary to obtain a clear picture.
e large deep-sea species, *E. barbata*, gave similar results to those obtained
h the seasonally nearest sample of *E. norvegica*, namely, that taken on
ctober 1950.

vadne nordmanni contained no detectable vitamin A in the quantity
mined, but information is lacking as to weight or numbers of this sample.
specimens of *Gammarus marinus*, weighing 14 g., produced no reaction
the vitamin, nor did 4 g. of the carrion feeder, *Orchomenella nana*.

tomical Distribution of Vitamin A and Carotenoids

oncurrently with studies of the quantitative aspects of vitamin A and
tenoids in marine Crustacea, attempts were also made to determine
ther these substances were locally concentrated or diffusely distributed
ughout the tissues of the animals. The first dissection was crude. Cooked
wns (*Leander serratus* (Pennant)) from the fishmonger were divided into
skeleton, cephalothorax and abdomen. Results of analyses showed a
min A concentration in the exoskeleton of 4·8 i.u./g.; in the cephalothorax,
i.u./g.; and in the abdomen, 0·1 i.u./g.

he observations of Wald (1941, 1943, 1945), Neilands (1947) and other
kers on the presence of vitamin A in the eyes of various Crustacea, and
eral knowledge of its importance as a factor in the visual cycle, drew
ntion to the eyes as possible sites of accumulation of vitamin A. In
ition, the eyes of euphausiids, which are rich in vitamin A, are bigger in
tion to the rest of the animal than those of other Eucarida in which the
min is present in much smaller amounts. Lönnberg (1934) tested extracts
n the eyes of several species of Eucarida and these gave, in every instance,
lue colour with antimony trichloride, especially intense with *Meganycti-*
nes norvegica. His technique, however, did not separate vitamin A and
otenoids.

phausiacea. At our request, Messrs Ash and Brachi, of the scientific staff
W.F.S. *Balaena*, divided some boiled specimens, weighing 20 g., of
phausia superba from the stomach of a whale into three anatomical groups,

soft parts, exoskeleton and eyes, subsequently preserving them in alcohol. The oil was extracted from each group and tested for vitamin A. That from the soft parts contained 6 i.u./g., or 5·6 % of the total vitamin A of the 20 g. sample, that from the exoskeleton 38 i.u./g., or 27·7 %, and that from the eyes 1000 i.u./g., or 66·7 %.

Separation of the eyes from the rest of the animals was then carried out in the northern euphausiids, *Meganyctiphanes norvegica* and *Thysanoessa raschii*, and the eyes contained 92–98 % of the total vitamin A present in the former and 82–98 % in the latter. Typical results of these experiments are shown in Table XIV. There was no marked variation in the percentage o vitamin A in the eyes throughout the series of size-groups. The high concentration of vitamin A in the oil from the eyes of the larger *Meganyctiphane* is particularly striking, since it may represent a content of up to 5 % pur vitamin A. It is noteworthy that a high proportion also of the total astaxanthi content of the animal is present in the eyes, the amounts being 20–50 % i *Meganyctiphanes* and 60–80 % in *Thysanoessa*.

TABLE XIV. DISTRIBUTION OF OIL PER CENT, VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN WHOLE ANIMAL (A) AND EYES (E) OF NORTHERN EUPHAUSIIDS OF DIFFERENT WEIGHTS

			Vitamin A			Astaxanthin			Vitamin A (Astaxanthi eyes as per of that in anim
Tissue	Wt. (mg.)	Oil	(i.u./specimen)	(i.u.)	(i.u./g. oil)	(µg./specimen)	(µg.)	(mg./g. oil)	*V*
				Meganyctiphanes norvegica (M. Sars)					
A	70	2·2	1·2	17	760	4·2	60	2·7	—
E	2	6·2	1·1	717	11,600	2·2	1,440	23	92
A	312	3·4	5·5	18	520	17	54	1·6	—
E	5	4·7	5·4	1,150	24,400	5·6	1,200	26	98
A	738	5·3	43	58	1,090	51	69	1·3	—
E	11	1·0	40	3,610	361,000	14	1,220	122	94
				Thysanoessa raschii (M. Sars)					
A	18	3·0	1·52	84	2,790	1·1	58	1·9	—
E	0·5	8·3	1·48	2,740	33,000	0·8	1,490	18	97
A	38	4·9	4·2	113	2,300	3·1	83	1·7	—
E	0·5	15	4·1	7,750	51,300	2·5	4,680	31	98
A	55	5·9	5·5	100	1,690	2·0	37	0·6	—
E	0·8	9·0	5·4	7,000	77,700	1·5	1,980	22	98

Further dissection of larger specimens of *Meganyctiphanes* was done i order to ascertain the location of the small portion of vitamin A not presen in the eyes. The anatomical groups examined and the results derived fro them are shown in Table XV. The body vitamin A appears to be abou equally divided between the exoskeleton and the contents of the cephalothora

Decapoda. The anatomical distribution of vitamin A in the Decapoda wa studied, and results obtained were similar to those given by the Euphausiace ough certain differences correlated with the smaller concentrations of the min present were noticeable. Cooked specimens of the common lobster *marus vulgaris* M.-E.) were examined at an early stage in this connexion, results for the whole animals are not available, since parts suitable for nan consumption had been retained by the catering firm who kindly plied the lobsters. In particular, the hepatopancreas was absent and so ults for comparison with those of Neilands (1947), who found that this an contained about one-third of the vitamin A present in the eyes in the

BLE XV. DISTRIBUTION OF OIL PER CENT, VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN *MEGANYCTIPHANES NORVEGICA* (M. SARS)

		Vitamin A			Astaxanthin		
	Oil (%)	(i.u./specimen)	(i.u./g.)	(i.u./g. oil)	(μg./specimen)	(μg./g.)	(μg./g. oil)
		Mean wt. of specimen, 386 mg.					
on	9·8	0·21	1·3	13	19	116	1,180
horax contents	34	0·16	2·7	7·9	11	187	545
contents	3·3	0	0	0	1·7	11	330
r)	11	14·3	2,460	22,400	11	1,950	17,700
imal	10	14·7	38	376	43	111	1,100
		Mean wt. of specimen, 513 mg.					
horax eton	6·6	0·7	5·3	80	14	100	1,520
exoskeleton	3·1	0·3	3·4	110	0·8	11	362
horax contents	9·2	0·8	7·8	85	6·6	63	680
contents	2·1	0·2	1·2	57	0·9	5·6	267
)	3·4	24	2,880	84,700	12	1,400	41,100
imal	5·1	26	55	1,080	34	73	1,430

erican lobster (*Homarus americanus*), are not yet available. Results for the ster and other decapods are given in Table XVI. Though most of these mals have vitamin A present exclusively in the eyes, *Crangon allmani* and *dalus bonnieri* are interesting in that the vitamin is present also in the ly, in amounts comparable with those found in bodies of *Meganyctiphanes* similar sizes. The great difference is in the eyes, which are relatively much ller in the Decapoda and contain less vitamin A, the concentration in *dalus* eyes, for instance, being only about one-tenth of that in those of *ganyctiphanes*. The eyes of lobsters contain about 90 % of their vitamin A he ester form, and a similar figure was obtained for the eyes of *Nephrops vegicus*.

BIOLOGICAL TESTS

Methods

paration of Material for Tests with Rats and Standards used

The method of extraction was as described earlier in this paper. The oil tained was diluted suitably with arachis oil and given directly to rats, or

TABLE XVI. DISTRIBUTION OF OIL PER CENT, VITAMIN A AND CAROTENOIDS PER SPECIMEN AND PER GRAM IN SOME DECAPODS

				Vitamin A			Carotenoids			
Part of animal	Date	Locality	Oil (%)	(i.u./specimen)	(i.u./g.)	(i.u./g. oil)	(μg./specimen)	(μg./g.)	(μg./g. oil)	β-Carotene (μg./g. oil)
Homarus vulgaris M.-E.										
Exoskeleton (anterior)	2. v. 50	—	1·0	0	0	0	10,500	48	4,800	60
Exoskeleton (posterior)	2. v. 50	—	2·0	0	0	0	1,550	72	3,600	44
Mixed internal organs*	2. v. 50	—	6·9	0	0	0	3,200	19	274	36
Ovaries	2. v. 50	—	16	0	0	0	—	151	911	100
Laid eggs	2. v. 50	—	14	0	0	0	—	139	1,010	154
Eyes (pair)	2. v. 50	—	1·6	11	23	1,410	35	75	4,700	59
Carcinus maenas (Pennant)										
Hepatopancreas	10. x. 50	Burnham-on-Crouch	2·0	0	0	0	57	33	1,640	368
Testes	10. x. 50	Burnham-on-Crouch	—	0	0	0	—	—	—	—
Eyes (pair)	10. x. 50	Burnham-on-Crouch	—	1·5	—	—	5	—	—	—
Crangon allmani Kinahan										
Body less eyes	21. xi. 50	Loch Fyne	1·5	0·3	0·5	31	2·9	4·4	293	None
Eyes (pair)	21. xi. 50	Loch Fyne	5·5	0·2	43	778	—	—	—	—
Cephalothorax exoskeleton	15. ii. 51	Loch Fyne	1·1	0·2	0·9	82	2·1	7·7	700	None
Abdomen exoskeleton	15. ii. 51	Loch Fyne	1·0	0·2	0·8	80	1·9	7·9	790	None
Cephalothorax contents	15. ii. 51	Loch Fyne	3·9	0·3	2·3	59	1·0	8·3	213	None
Abdomen contents	15. ii. 51	Loch Fyne	1·1	0	0	0	0·7	2·0	182	None
Eggs from 'berried' females	15. ii. 51	Loch Fyne	7·2	—	0	0	—	12	171	None
Eyes (pair)	15. ii. 51	Loch Fyne	12	0·1	28	231	0·4	75	631	None
Eupagurus bernhardus L.										
Body, less eyes	10. x. 50	Burnham-on-Crouch	3·6	0	0	0	178	43	1,200	38
Eyes (pair)	10. x. 50	Burnham-on-Crouch	0·6	0·5	23	103	0·5	46	8,250	None
Nephrops norvegicus L.										
Body, less eyes	13. ii. 51	Loch Fyne	0·6	0	0	0	1,840	11	1,790	4
Eyes (pair)	13. ii. 51	Loch Fyne	0·3	7·7	14	4,590	6·8	12	4,060	None
Eggs from 'berried' females	20. xi. 50	Loch Fyne	6·5	—	0	0	—	27	421	34
Pandalus bonnieri Caullery										
Average weight of specimens 4·9 g.										
Body, less eyes	15. ii. 51	Loch Fyne	1·6	2·4	0·5	31	224	46	2,870	None
Eyes (pair)	15. ii. 51	Loch Fyne	0·8	4·8	92	11,500	1·1	21	2,600	None
Average weight of specimens 1·7 g.										
Body, less eyes	15. ii. 51	Loch Fyne	1·5	1·0	0·6	40	35	20	1,350	None
Eyes (pair)	15. ii. 51	Loch Fyne	1·0	2·0	91	9,100	0·8	37	3,720	None.
Spirontocaris spinus (Sowerby)										

ected to chromatography to remove the bulk of the pigments, or saponi- and chromatographed as for the chemical test, and the non-saponifiable due containing vitamin A in the alcohol form was diluted with arachis oil feeding to rats. For the rat-growth tests, the oils were kept under nitrogen ne refrigerator and tested periodically for vitamin A by the Carr-Price test. or the liver-storage test, the standard was a fish-liver oil at 106,000 i.u./g., for the rat-growth experiments the International Standard for vitamin A 0,000 i.u./g. in cottonseed oil was used. Both were diluted suitably for ling with arachis oil stabilized with hydroquinine.

paration of the Rats

Hooded Norwegian rats of our own breeding were used. Rats partly cient in vitamin A were prepared exactly as described by Thompson *et al.* 9). It will be recalled that such rats have vitamin A neither in the liver in the intestine, but have not stopped growing.

o obtain rats wholly deficient in vitamin A the mothers were deprived milk, liver and carrots, their dietary sources of vitamin A, as soon as young were born; from the 16th day of lactation they were given our min A-deficient diet (Henry, Kon, Mawson, Stanier & Thompson, 1949). young were weaned on to this diet at 21 days of age and continued on it ing the 'running-out' and dosing periods.

artly deficient rats were dosed by the method described by Thompson *l.* (1949), except that actual or presumptive quantities of the order of , 50 and 25 i.u. of vitamin A from the standard or the oils under test e added to 400 mg. of arachis oil.

xperiment 1. In a preliminary experiment, rats were given the vitamin A hol chromatographically separated from the non-saponifiable residue of obtained from krill forming the stomach contents of fin whale 1 (see le II), consisting mainly of *Euphausia superba*. They were anaesthetized r 3 hr., and the intestinal contents were washed out *in vivo*; the intestinal l and contents and the liver were then analysed as described by Thompson, ude, Coates, Cowie, Ganguly & Kon (1950).

xperiment 2. The non-saponifiable residue from oil of the same source as Exp. 1 was diluted with arachis oil to a concentration of approximately i.u./g. Three rats were used for each of three levels of standard or krill and were killed after 3 hr., the livers being removed for analysis.

Using wholly deficient rats the following rat-growth tests (Booth, Kon & lam, 1934) were performed.

xperiment 3. Groups of eight (four of each sex) deficient rats were dosed he rate of 2 or 4 i.u. International Standard Vitamin A daily or with the -saponifiable residue of the body oil of *Meganyctiphanes norvegica*, *ysanoessa raschii* and *Euphausia superba*; these were suitably diluted with arachis oil and given to the rats to supply 2 and 4 i.u. vitamin A as determined chemically. The rats were dosed twice a week.

Experiment 4. Groups of fourteen (seven of each sex) deficient rats were dosed twice weekly, at the rate of 1, 2 and 4 i.u. daily with vitamin A standard or the non-saponifiable residue, dissolved in arachis oil, of the oil of *Meganyctiphanes norvegica* caught in Loch Fyne in January 1950.

Experiment 5. Groups of ten (five of each sex) deficient rats were used. Oil from *Thysanoessa raschii*, caught in Loch Fyne in January and August 1950, was tested intact or after chromatographic removal of the pigment; these oils were fed undiluted at levels supplying about 1, 2 and 4 i.u. vitamin A daily. The standard was also fed at these levels. Animals were dosed twice a week as in the other experiments.

Results

Experiment 1. The vitamin A in the intestinal wall of the rats was largely in the ester form, while in the liver both forms of vitamin A were present in roughly equal amounts. There was no vitamin A in the gut contents. The behaviour of the vitamin A from krill was, therefore, exactly the same as of that formed from β-carotene or of pure vitamin A, when given under similar conditions (cf. Thompson *et al.* 1950).

Experiment 2. The results of this experiment are given in Table XVII. In Fig. 6 the net vitamin A* stored in the liver is plotted against the dose o vitamin A for the standard. It will be noticed that the points lie on a straigh line which does not pass through the origin. This curve can be used to esti mate the potency of the krill oil by reading off, against the net liver stores o vitamin A, the corresponding amount of vitamin A in each dose.

These results show a strong similarity in the behaviour of vitamin A from krill and from fish-liver oils, though naturally storage in the liver cannot b taken as a decisive biological proof of the identity of the stored substanc with vitamin A.

Experiment 3. Table XVIII shows the results of this experiment an indicates that the chemical potency was two to three times higher than th biological, despite the findings that the euphausiid vitamin A was chemicall and physically indistinguishable from pure vitamin A.

Experiment 4. Table XIX shows that, even with the larger number of rat used and a more satisfactory test, the chemical result was almost twice th biological.

Experiment 5. The discrepancy between the chemical and the biologica potencies is again apparent in Table XX, which also indicates that th pigments (astaxanthin) did not affect the biological activity, since there was n difference between results obtained with whole oil and those with whole o less pigments. This also confirms biologically the absence of β-carotene o other precursors of vitamin A from the pigments.

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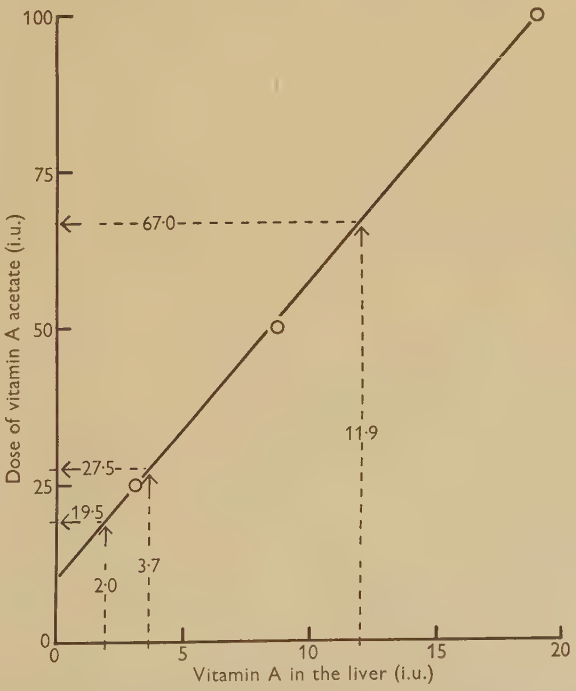
* Net vitamin A = vitamin A stored in liver less vitamin A found in control livers.

LE XVII. EXP. 2. VITAMIN A, ALCOHOL AND ESTER, AS I.U. PER ORGAN, IN LIVER OF PARTLY VITAMIN A-DEFICIENT RATS DOSED WITH 0·4 G. ARACHIS OIL CONTAINING VITAMIN A FROM FISH-LIVER OIL (B) OR KRILL OIL (C)

Dose (i.u.)	Rat no.	Alcohol	Ester
100 B	1	14·2	9·1
	2	12·1	13·8
	3	10·8	7·7
	Mean:	12·4	10·2
50 B	4	7·3	5·2
	5	8·1	6·0
	6	5·2	5·2
	Mean:	6·9	5·5
25 B	7	3·6	3·4
	8	3·3	3·4
	9	3·3	3·6
	Mean:	3·4	3·5
79 C	10	4·3	8·6
	11	7·2	8·6
	12	8·2	9·9
	Mean:	6·6	9·0
39·5 C	13	3·7	3·0
	14	4·5	4·3
	15	3·5	3·2
	Mean:	3·9	3·5
19·8 C	16	3·0	2·6
	17	3·2	2·8
	18	2·8	2·8
	Mean:	3·0	2·7

Mean for two control rats: 1·0 alcohol, 2·7 ester.



. 6. Exp. 2. Liver storage tests with rats given the non-saponifiable residue of euphausiid oils. Graph showing relationship between dose of pure vitamin A acetate and vitamin A stored in the liver, and the calculation of doses of vitamin A corresponding to storage of vitamin A found in test rats.

Table XVIII. Exp. 3. Chemical (antimony-trichloride) and Biological Tests on Extracts from Euphausiid Oils fed to wholly Vitamin A-deficient Male Rats, in i.u. per gram

		Biological	
	Chemical	Value	True fiducial limits ($P=0·95$)
M. norvegica	147	55·7	5·4 to 113·6
T. raschii	148	64·3	4·5 to 227·3
E. superba	158	71·3	9·9 to 138·5
Standard preparation of vitamin A	228	—	—

Table XIX. Exp. 4. Chemical (antimony-trichloride) and Biological Tests on Extracts from Euphausiid Oils fed to wholly Vitamin A-deficient Rats, in i.u. per gram

		Biological	
	Chemical	Value	True fiducial limits ($P=0·95$)
M. norvegica	378	—	—
♀ Rats	—	234	153 to 390
♂ Rats	—	307	192 to 612
Standard preparation of vitamin A	174	—	—

Table XX. Exp. 5. Chemical (antimony-trichloride) and Biological Tests on Euphausiid Oils fed to wholly Vitamin A-deficient Rats, in i.u. per gram

		Biological	
	Chemical	Value	True fiducial limits ($P=0·95$)
T. raschii: Whole oil	678	—	—
♀ Rats	—	333	204 to 632
♂ Rats	—	391	249 to 730
T. raschii: Whole oil less pigment	610	—	—
♀ Rats	—	332	200 to 645
♂ Rats	—	443	279 to 869
Standard preparation of vitamin A	169	—	—

Discussion

The study of the various groups of Crustacea reported here, though not covering so far all the classes, has revealed marked differences in content and concentration of vitamin A. In the lower Crustacea, the representatives of the Copepoda and Cladocera so far examined contained no measurable vitamin A, nor did those of the Peracarida. In the Eucarida in genera vitamin A was present, with a marked difference between the two orders in that Euphausiacea were by far the richer. Why should the vitamin, rising sharply in concentration with the size of the animal, be found in this order in such relatively enormous quantities? Why, moreover, should it be almost exclusively present in the eyes? Is it connected in these animals with some special visual function, has it some other physiological duty, or are the eyes merely storage organs for something produced or accumulated in excess capods are predominantly benthic in their habits, and perhaps in their stant contact with the sea floor they have less need for acute vision than free-swimming euphausiids. Admittedly other free-swimming Crustacea h as copepods contain no vitamin A, but their reaction to light may be of ifferent character from that of euphausiids, and in any case the structure of ir eyes is much simpler. In fact, the exceptionally large and prominent s are a characteristic feature of euphausiids which may be specially well pted for adequate vision in the low intensity of light at the depth they quent. It will be recalled that a large specimen of *Meganyctiphanes vegica* may contain in its eyes some 40 i.u. of vitamin A, and even allowing the special demands of this order it seems hard to believe that vitamin A needed there in such quantities solely for the functions of vision. In *ganyctiphanes* the concentration of vitamin A in the eyes is in the range 0–12000 i.u./g. dry weight (taking the water content as 70 %). With se figures may be compared the findings of Wald (1935) that mammalian inas have vitamin A concentrations of about 70 i.u./g. dry weight and frog inas have 1200 i.u./g., whereas the pigmented layers of the frog eye ntain 6000 i.u./g. Morton & Rosen (1949) give results for seasonal varia-ns of vitamin A in frog eyes. The highest value they obtained was about μg./eye; in a frog of 20 g. weight of which the eyes account for about % this gives a vitamin A concentration of 105 i.u./g. wet weight, or proximately 300 i.u./g. on the dry basis.

We intend to investigate the distribution and anatomical location of amin A in euphausiid eyes in the hope of finding thereby something more out its purpose there. In the meantime the possibility of its being to some tent an excretory substance cannot be dismissed.

Be it as it may, it is evident that baleen whales can derive from krill mense quantities of vitamin A, quite sufficient to account for the great res of the vitamin in whale liver. According to Einarsson (1945) a large ale may have up to 1200 l. of krill in its stomach. This would be roughly ton, and with a vitamin A potency of, say, 20 i.u./g. would yield about 00,000 i.u.

The richness in vitamin A of the food of baleen whales associated with the gh concentration of vitamin A in their livers may be contrasted with its lack another crustacean, *Calanus finmarchicus*, and the corresponding paucity vitamin A of animals whose principal food it forms, the herring and the sking shark.

In the euphausiids studied by us vitamin A was accompanied in the eyes astaxanthin in high concentrations and in fact a large proportion (20–50 % *Meganyctiphanes norvegica* and 60–80 % in *Thysanoessa raschii*) of the total gment of these animals was present there. The function of this characteristic gment of Crustacea is not yet understood. Its value, if any, for the higher imals, preying on Crustacea, presents an interesting problem deserving further study. Thus Wald (1945) found astaxanthin in the eyes of birds; Grangaud & Massonet (1950) report that it prevents and cures xerophthalmia in vitamin A-deficient rats, though it does not alleviate other signs of the deficiency or promote growth, and it is well possible that the pigment has some vitamin-like function. Its chemical constitution is such that it can hardly act as precursor of vitamin A itself. Furthermore, in our biological tests it proved entirely inactive in this respect. As corroborative evidence may be cited the fact that *Calanus finmarchicus* is at times quite rich in the pigment without apparently affecting the low vitamin A content of herring.

Again, we found no β-carotene in euphausiids, observations sharply at variance with those of Wagner (1939), who reported large quantities of β-carotene in northern krill, and we are entirely at a loss to understand how he came to detect this pigment. The finding of vitamin A in large quantities in certain euphausiids accounts, we hope adequately, for the immediate source of the vitamin A of the whale. It makes it also clear that baleen whales obtain most of their vitamin A as such and have no need and, indeed, no opportunity, to form it from precursors, though in common with other mammals they no doubt are able to do so. One link of a food chain is thus accounted for, but the fundamental problem of the site of origin of the vitamin A of marine organisms remains unexplained. The focus of attention has been moved from the whale to its food, and the question arises whether euphausiids in turn obtain their vitamin A preformed or whether they manufacture it themselves from precursors in their food.

Though our work on vitamin A in marine animals began more than two years ago, the more systematic study has been going on for little more than a year. In so short a period variations due to seasonal and other causes could be observed only in a very broad outline.

The relationship between size and vitamin A concentration in *Meganyctiphanes* stands out so clearly as to indicate close correlation. At first, size and content increase *pari passu*; with large animals the concentration also rises steeply. This may well indicate a change in food or metabolism around the weight stage of about 0·4 g.

Our data are, so far, insufficient to indicate seasonal variations in vitamin A content of *Meganyctiphanes*, especially as the size of the animal exerts such marked influence, but with *Thysanoessa raschii* the indications are clearer that the concentration of vitamin A may be higher during the winter months. Our evidence so far is that *Thysanoessa* differs from *Meganyctiphanes* in that concentration of vitamin A does not increase with size.

The isolated observation of the relation between vitamin A content and depth of haul in *Thysanoessa inermis* is striking, especially since the animals were of uniform size and *Meganyctiphanes* taken in the same hauls, also of uniform size, did not exhibit this variation.

In the decapods studied vitamin A was also largely concentrated in the eyes, ıgh in some species, of which *Crangon allmani* is the notable example, east half of the vitamin was present in other parts of the body. We do know yet whether in these animals it was adventitious in the alimentary ai or as a true constituent. We are also in a similar doubt about the ill quantity of vitamin A found in euphausiids not in the eyes.

'he values quoted for vitamin A throughout this paper are those derived n chemical and physical measurements. We are satisfied that these were e with the necessary care and after adequate purification and separation, we are aware that so far all our biological tests have indicated a potency er than that of corresponding laboratory measurements. This difference ts for all species studied regardless of the purity of the preparations fed, suggests either the presence of growth-inhibiting substances closely ciated with the vitamin A fraction or the presence in these purified tions of chemically related substances giving rise to an artefact.

'he work described in this paper has been supported by a grant from the 'elopment Commission. Its success so far has largely depended on the ly assistance given by many people engaged in marine biological work in the whaling industry, whom we have from time to time visited either discussion or for the use of facilities they control. The unique facilities collecting euphausiids in Loch Fyne have meant that the Marine Station Aillport has had much more than a fair share of our visits, and we are emely grateful to Mr E. Ford and his staff, especially Drs S. M. Marshall, '. Orr and D. T. Gauld, for all their help and forbearance. We must also ıtion Dr C. E. Lucas and his staff at the Fisheries Laboratory, Aberdeen; J. A. Lovern of the Torry Research Station, Aberdeen; Dr J. G. Sharp he Low Temperature Research Station, Cambridge; Messrs Blomvåg ıl, their manager, Mr A. Hojem and Capt. H. Harneshaug of their catcher, *ıl* 2, in Norway; Messrs Scottish Whalers Ltd., and their manager, ot. H. Jespersen, at West Loch, Tarbert, Harris; Messrs United Whalers . and their chemists, Mr C. E. Ash and Mr R. M. Brachi, of W.F.S. *aena*; Messrs M. Graham and R. S. Wimpenny of the Fisheries Laboratory, vestoft; Mr F. S. Russell and his staff at the Marine Laboratory, nouth; Mr R. D. Waugh and his staff at the Oyster Research Station, nham-on-Crouch; Drs N. A. Mackintosh and H. Bargmann and Mr R. ·ke and other members of the staff of the Discovery Investigations; f. C. H. O'Donoghue, Dr N. B. Eales and Mr M. I. Crichton of Reading versity; and Dr E. B. Hughes and Mr D. H. F. Clayson of Messrs Lyons' oratories, Hammersmith.

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SUMMARY

Planktonic, benthic and littoral Crustacea were collected from localitie around the British coast, from Norwegian and Faeroese waters and from th Antarctic, and their content of preformed vitamin A and carotenoid pigment was measured.

Methods are described for the preservation of specimens, the extractio and separation of vitamin A and carotenoids and the measurement of vitamin A by chemical, physical and biological tests, and of carotenoids by physica tests.

Free-swimming euphausiids were found to contain, in addition to larg quantities of astaxanthin, high concentrations of preformed vitamin A, but n β-carotene.

Krill, taken from the stomachs of whales, consisting of *Meganyctiphane norvegica* in arctic waters and *Euphausia superba* in antarctic waters, als contained no β-carotene, but preformed vitamin A was present, although i lower concentrations than in free-swimming animals.

In *Meganyctiphanes norvegica*, the vitamin A concentration increased wit the size of the animal, but in *Thysanoessa raschii* it was unchanged. In bot species, the astaxanthin content, but not concentration, was higher in th larger animals.

The eyes of the euphausiids contained over 90 % of their total vitamin A the rest being in the exoskeleton and contents of the cephalothorax. A hig proportion of the total astaxanthin was also in the eyes.

The vitamin A concentration was much lower in the decapods, the difference being in the eyes, since their bodies contained the vitamin in quantitie comparable to those found in the bodies of euphausiids.

Vitamin A was absent from the amphipods, isopods and Cladocer examined. In the Copepoda, *Calanus finmarchicus* was devoid of the vitamin but it was present in small quantities in some samples of *Euchaeta norvegic*

Biological tests with rats on oils or concentrates from euphausiids indicate potencies about one-half those expected from results of chemical and physic tests. The pigments showed no biological activity.

It is suggested that the high vitamin A content of euphausiids forming th food of whales is adequate to account for the rich liver stores of the vitami in those mammals.

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OTES ON THE GROWTH AND BIOLOGY OF THE PRAWN *PANDALUS BONNIERI* CAULLERY

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(Text-figs. 1 and 2)

ndalus bonnieri Caullery has for a long time been known to occur in con- erable numbers in the deeper waters of the Clyde Sea Area. Its life history growth have been investigated, with special reference to sexual develop- nt, since the genus *Pandalus* contains not only species that are dioecious, also those that are protandrous.

he author expresses indebtedness to the skipper and crew of M.V. *Calanus* their unfailing help in collecting the material, and to Miss E. R. Wallace assistance in measuring specimens. Thanks are also due to Dr A. Ritchie he Fisheries Laboratory, Aberdeen, for supplying records of the occurrence *P. bonnieri* on the west coast of Scotland and in the North Sea, and to eagues at the Marine Station for assistance in preparing this paper.

Collection of Material and Data

adult prawns were caught in the Firth of Clyde on trawling grounds off Island of Bute (National Grid Reference 26/1159–1162) at a depth of –90 m. The gear used was an 8 ft. Agassiz trawl having a net mesh of ½ in. , which was found to retain prawns above 7 mm. carapace length (3·7 cm. al length). The duration of a haul was 15 min., and such hauls were eated until a sufficient number of specimens had been obtained for the y's sample.

For catching prawns smaller than 7 mm. carapace length, a 2 m. stramin was fixed to a small beam trawl, so modified that the net worked about n. above the muddy bottom. Early post-larval stages were seldom obtained any numbers.

All specimens above 5 mm. carapace length were measured with fine lipers in the fresh condition and then fixed in 10% formalin for subsequent eservation in 70% alcohol.

The standard measurement made was the 'length of the carapace', being e distance from the posterior margin of the eye socket to the dorsal posterior margin of the carapace. Measurement was made to the nearest 1 mm. below, and the data were recorded in 1 mm. groups.

An approximation to the *total* length, including the rostrum, can be obtained by multiplying the *carapace* length in the male by 5·2, and in the female and juvenile by 5·4. In both males and females of carapace length exceeding 17 mm., however, the total length calculated in this manner works out on the high side.

Geographical Distribution

P. bonnieri Caullery is essentially a deep-water prawn and, like *P. borealis* Krøyer, is usually found at a depth of about 100 m. Its distribution extends from Iceland (Stephensen, 1939, p. 22) to the Bay of Biscay (Caullery, 1896). It does not appear to be very plentiful round Iceland or to enter Greenland waters. From Norway it is recorded by Wollebaek (1908) from several localities in both the south and west. Dr Ritchie has kindly supplied me with sixty records of its occurrence in the North Sea and from the west coast of Scotland. These records show that it is taken frequently in the North Sea from the Shetland Isles to Peterhead, and on one occasion was taken as far south as 56° 03′ N., 1° 45′ W. The majority of specimens were obtained beyond the 100 m. line along the western edge of the deep central basin of the North Sea, but they were not abundant at any locality. On the west coast of Scotland it is also taken beyond the 100 m. line and appears to be generally distributed, the greatest numbers being obtained off the Butt of Lewis and south of South Uist. In general, records are absent for the north of Scotland and west of the Outer Hebrides, but the prawn has been obtained on two occasions west of the Orkneys. Dr Ritchie suggests that their absence from these areas may be due to the texture of the sea-floor in those localities. Calman (1899) was the first to record it from the Firth of Clyde. He also records it from the west coast of Ireland and off Rockall. Kemp (1910) states that it is abundant in the Irish Sea and off the west coast of Ireland, but it has not so far been taken off the south of Ireland. It is not recorded in the *Marine Fauna of the Isle of Man* (Moore, 1937), but Mr N. S. Jones of the Marine Biological Station, Port Erin, informs me that he has taken it frequently since 1946 on muddy bottom in 90–130 m., 7–11 miles north-west of Bradda Head. It does not appear in the *Plymouth Marine Fauna* (Mar. Biol. Assoc., 1931). Lebour (1940), however, records the larvae as occurring in plankton off Seven Stones Lightship, Scilly Isles (50° 03½′ N., 6° 04½′ W.)

Occurrence in the Clyde

In the Clyde *P. bonnieri* is taken frequently in water as shallow as 40 m. although it is most abundant at depths of 90–130 m., on grounds composed of soft grey mud, inhabited by an animal community similar to that associated with *P. borealis* as described by Hjort & Ruud (1938).

he commonest bivalve molluscs are *Abra alba* (Wood) and various species *ucula*, of which *N. nucleus* (L.) and *N. sulcata* Bronn are the commonest. *asira flexuosa* (Montagu) is, however, rare and *Chlamys septemradiata* iller) is not very abundant on this ground. Among the annelids, *Pectinaria ica* (Pallas), *Lipobranchius jeffreysii* (McIntosh) and *Glycera rouxi* Audouin M.-Edwards are all very plentiful. Of the Crustacea, *Spirontocaris us* var. *lilljeborgi* (Danielssen), *Crangon allmani* Kinahan and *Nephrops vegicus* L. are the commonest species.

MIGRATION

keley (1931) drew attention to evidence that larvae of pandalids meta- phose in shallow water, and Poulsen (1946) also found young *Pandalus alis* in shallow water round Denmark. In the Clyde the normal prawn unds, and the shallow water areas in the neighbourhood, were trawled a bottom stramin net (see collection of material and data) during June July 1951 for early metamorphosed prawns. These were not obtained in numbers until 31 July at a depth of 130 m. No information was therefore ained on the movements of young *P. bonnieri*.

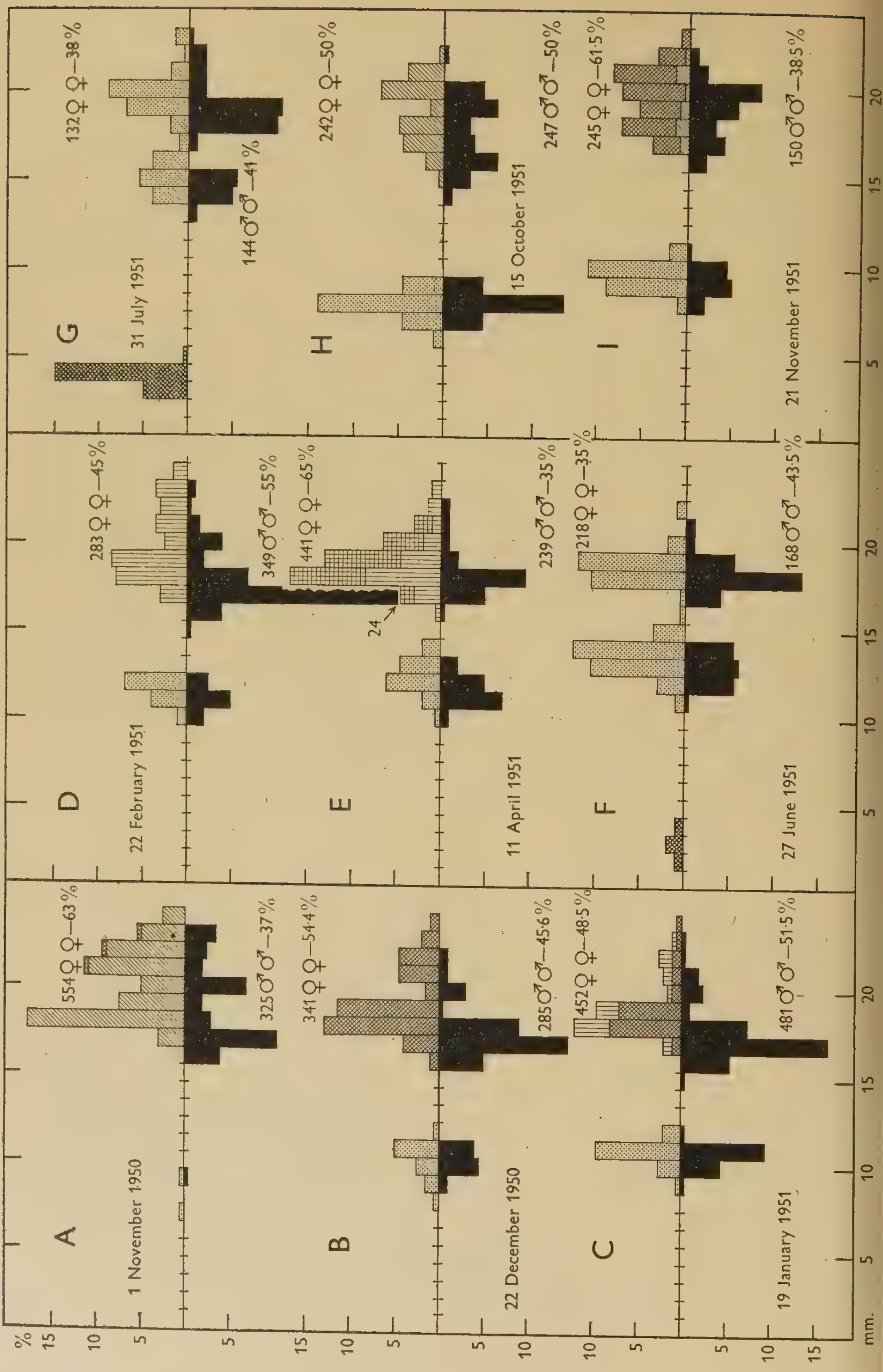
dult prawns could not be found during August and September 1951 in Clyde. According to Hjort & Ruud (1938) *P. borealis* rises off the bottom ng dull weather and during the hours of darkness. A similar habit in *onnieri* might account for the difference in numbers on successive days, not for the scarcity of specimens during two months. It is possible that h the young and adults moved into the nearby inshore boulder-strewn unds where trawling was impossible.

BREEDING

he Clyde the ovaries of adult prawns begin to mature in July, when they w through the carapace as a greenish mass. In September the fully ripe ries darken to a deep sage green. Egg-laying begins at the end of October h the larger size-groups laying first (Fig. 1A), and continues for about weeks. By the end of December the whole adult female population is in ry (Fig. 1B).

he eggs when first laid are a beautiful deep green colour and oval in shape 2 × 1·5 mm.). The sage-green colour of the newly laid egg in *P. bonnieri* is contrast with the blue-green egg of *P. borealis*, the light emerald green of *montagui* Leach and the fawn colour in *P. propinquus* G. O. Sars.

he green colour gradually disappears as the larvae develop, and just before ching takes place the green is almost replaced by pale violet and yellow mentation with the prominent red chromatophores of the thoracic region arly showing. The eye pigmentation develops gradually and is first seen er about 3 months' incubation.


A
1 November 1950
554 ♀♀—63%
325 ♂♂—37%
B
22 December 1950
341 ♀♀—54·4%
285 ♂♂—45·6%
C
19 January 1951
452 ♀♀—48·5%
481 ♂♂—51·5%
D
22 February 1951
283 ♀♀—45%
349 ♂♂—55%
E
11 April 1951
441 ♀♀—65%
239 ♂♂—35%
24
F
27 June 1951
218 ♀♀—35%
168 ♂♂—43·5%
G
31 July 1951
132 ♀♀—38%
144 ♂♂—41%
H
15 October 1951
242 ♀♀—50%
247 ♂♂—50%
I
21 November 1951
245 ♀♀—61·5%
150 ♂♂—38·5%
%
15
10
5
mm.
5
10
15
20

he number of eggs laid by *P. bonnieri* increases from approximately 1000 6 mm. carapace length to 4000 at 24 mm. carapace length. Within any n millimetre size-group the variation appears to be *c.* ± 10%.

n 1951 the first larvae were liberated in the third week in February, ough the number of prawns that had completed incubation was too small e recorded on the graph for 22 February (Fig. 1 D). The total hatching od extended over 2 months and all the larvae were liberated by the end pril (Table I).

is probable that moulting and the loss of the ovigerous setae take place in 10 days of the liberation of the larvae from the eggs, but the period is known. Höglund (1943) has described and illustrated the ovigerous setae *P. borealis* and shown the arrangement to be similar for *P. bonnieri*.

TABLE I. BREEDING CONDITION IN SECOND- AND THIRD-YEAR *PANDALUS BONNIERI*

Date (1950–1)	Ovary ripe (%)	Eggs (%)	Eggs with eye pigmentation (%)	Larvae liberated (%)
15 Oct.	100	—	—	—
26 Nov.	22	78	—	—
6 Dec.	3	97	—	—
19 Jan.	—	60	40	—
22 Feb.	—	—	98	2
19 Mar.	—	—	90	10
11 Apr.	—	—	54	46
5 May	—	—	—	100

SEXUAL DIFFERENTIATION IN *PANDALUS BONNIERI*

r one whole year, the percentage of males in the catches has varied veen 35 and 55%, about a mean of 45%, and that of the females between nd 65%, about a mean of 55%. The sex was determined by the examina- of the pleopods under the binocular microscope. The sex characters of *onnieri* agree very closely with those given by Wollebaek (1908) for *orealis*. Among the males no transitional form of pleopod corresponding at illustrated by Jägersten (1936) for *P. borealis* was found.

any male prawns were dissected, but not one was found to possess ucts as well as vasa deferentia. Sections of the male gonad in prawns aged o months have been prepared, from which it could be seen that the gonad

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Legend to Fig. 1.

1. The percentage size distribution of *Pandalus bonnieri* in the Clyde Sea Area during 1950–51. Females above and males below the zero line. To the left the date of collection. To the right the number of females and males measured in each sample in total and in percentage of the whole sample. The juvenile population in Fig. 1 is included in the total percentage although captured with a different gear (see p. 259). In the females the state of sexual maturity is also shown.

was composed entirely of testicular tissue with no trace of ovarian tissue su as was found by Jägersten in *P. borealis*.

I therefore think *P. bonnieri* is completely dioecious. This finding is co firmed by Dr Ritchie in a personal communication.

RATE OF GROWTH IN *PANDALUS BONNIERI*

The data obtained on growth are shown in graphic form in Figs. 1 and 2 a summarized in Table II.

In preparing Fig. 2 the graph relates to modal lengths and not to arithme means as were used by Hjort & Ruud (1938) for *P. borealis*, by Höglund (194 for *Leander squilla* (L.) and by Forster (1951, *a*, *b*) for both *L. squilla* a *L. serratus* Pennant.

TABLE II. GROWTH OF *PANDALUS BONNIERI*

	O-Group		I-Group		II-Group	
Month of year	Age in months	Carapace length (mm.)	Age in months	Carapace length (mm.)	Age in months	Carapace length (mm.)
Apr.	0–2	0·9–1·2	12–14	♂ 11–12 ♀ 12	24–26	♂ 18 ♀ 18–19
June	2–4	2·5–3·0	14–16	♂ 12–14 ♀ 13–14	26–28	♂ 18 ♀ 18–19
July	3–5	3–5	15–17	♂ 14–15 ♀ 15	27–29	♂ 18–19 ♀ 19–20
Oct.	6–8	♂♀ 7–9	18–20	♂ 16 ♀ 17–18	30–32	♂ 19 ♀ 20
Nov.	7–9	♂♀ 9–10	19–21	♂ 17 ♀ 18	31–33	♂ 20 ♀ 21–22
Dec.	8–10	♂♀ 10–11	20–22	♂ 17 ♀ 18–19	32–34	♂ 20 ♀ 21–22
Jan.	9–11	♂♀ 10–11	21–23	♂ 17 ♀ 18–19	33–35	♂ 20 ♀ 22
Feb.	10–12	♂ 11 ♀ 12	22–24	♂ 17 ♀ 18–19	34–36	♂ 20 ♀ 21

On hatching in March and April the larvae have a carapace length 0·9 mm. (total length 4·2 mm.), and by the end of June, now from 2 to months old, they have metamorphosed and have a carapace length 2·5–3 mm. Metamorphosis takes place at about 2·2 mm. carapace leng By the end of July they have increased their carapace length from 3 to 5 mr and are still juvenile, with the sexes undifferentiated. At 5 mm. carapa length it becomes possible to distinguish the sexes by the shape of t pleopods, but the developing males at this early stage are less read

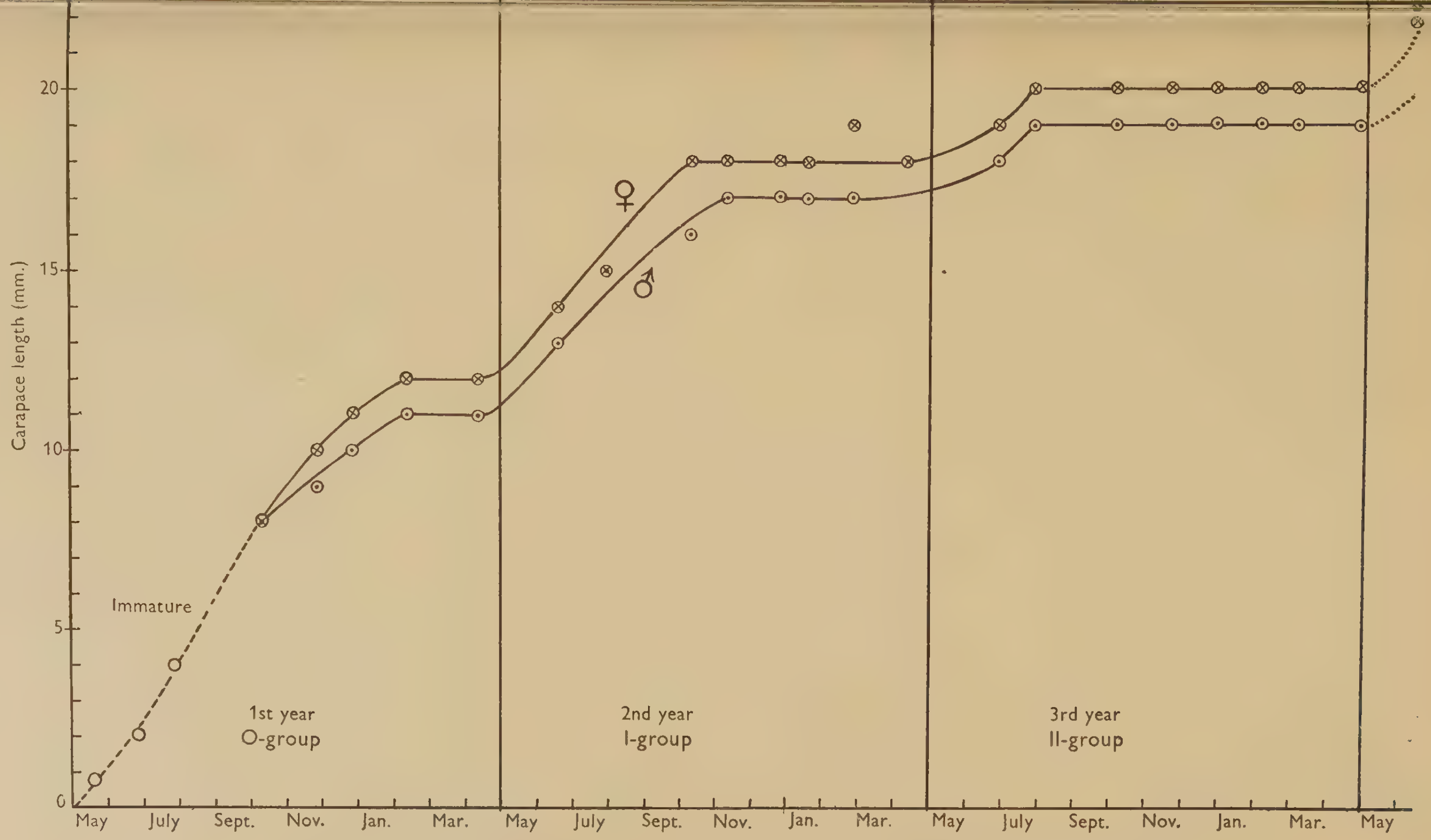

Fig. 2. *Pandalus bonnieri*. Modal lengths of various samples of three year-groups, forming an approximate growth curve.

separated than females from the undifferentiated juveniles because of a mor gradual transition.

The course of subsequent growth is shown in Fig. 2 and Table II. It wil be seen that the females are always larger than the males of a similar age Sexual maturity is reached at the age of 18–20 months (i.e. October) when th average carapace length in the males is 16 mm. and in the females 17–18 mm It seems possible, however, that the males reach maturity somewhat i advance of the females.

A few prawns live on to a fourth year, reaching a carapace length c 23–24 mm. but the numbers are very small and insufficient for inclusion.

SUMMARY

Pandalus bonnieri is a dioecious species and no protandrous hermaphrodite have been seen.

The breeding season and incubation period for *P. bonnieri* are described.

The number of eggs laid increases with the size of the female. At 16 mm carapace length the number of eggs is *c.* 1000 and at 24 mm. carapace lengt the number of eggs is *c.* 4000. The number for a single mm. size group varie roughly by ±10%.

Both male and female *P. bonnieri* become sexually mature at abou 18 months, and in the Clyde seldom live beyond 3 years of age.

The colour of the eggs in *P. bonnieri* is sage green, in *P. borealis* blue gree in *P. montagui* light emerald green and in *P. propinquus* fawn.

Juvenile *P. bonnieri* can be separated into males and females by the shap of the pleopods within 4–6 months of hatching.

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A NOTE ON THE BREEDING SEASON, SEX RATIO AND EMBRYONIC DEVELOPMENT OF THE DOGFISH *SCYLIORHINUS CANICULA* (L.)

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(With an Appendix by C. A. Hoodless and G. A. Steven)

(Text-figs. 1–3)

many years past the Department of Zoology at Bristol has obtained all its fish from a single supplier, Mr E. G. Williams of Ilfracombe. In addition hose fish purchased for dissection, a large number has been obtained for k on the development of the behaviour pattern in the embryo, and over last 3 years a certain amount of detailed but incidental information has n accumulated on the topics which form the title of this paper. This rmation supplements the interesting account given by Ford (1921), and e it is not proposed to carry out a more detailed study on these subjects, material is offered in its present form as a short additional chapter on the ogy of a common laboratory type, and in the hope of stimulating further k on these lines.

n addition to noting the numbers and sex of all dogfish delivered at the artment, all well-developed eggs are removed from the oviducts of the ales and are reared in the laboratory aquarium, where a very high propor- of the embryos (70–100 % of those received in the cooler months of the r) develop successfully. There is no reason to believe that the fish received other than random samples of the population caught, and the necessarily ative deductions made below are based on this assumption.

THE BREEDING SEASON

d (1921) presents evidence which suggests that eggs are laid throughout year. This may well be true, but the Ilfracombe fish show a rather more rply defined breeding season than the Plymouth material. No consign- nts have ever been delivered in the month of August, but for the remaining nths of the year the number and percentage of females carrying egg-purses hown in Table I and Fig. 2. The deliveries covered a continuous period n May 1949 to December 1951.

While the figures for some months are smaller than one could wish, they ear to show that breeding starts in November when only 18 % of the females are carrying egg-purses, and continues at least until July. From December to July any sample examined will show about one-third of the females carrying egg-purses, and this proportion remains remarkably constant

TABLE I

Month	No. of fish delivered			No. of females carrying egg-purses
	Male	Female	Total	
Sept.	7	50	57	0
Oct.	25	44	69	0
Nov.	83	197	280	35
Dec.	49	145	194	41
Jan.	7	172	179	73
Feb.	31	47	78	16
Mar.	115	186	301	62
Apr.	71	61	132	20
May	228	158	386	47
June	119	61	180	19
July	8	34	42	13
Aug.	—	—	—	—

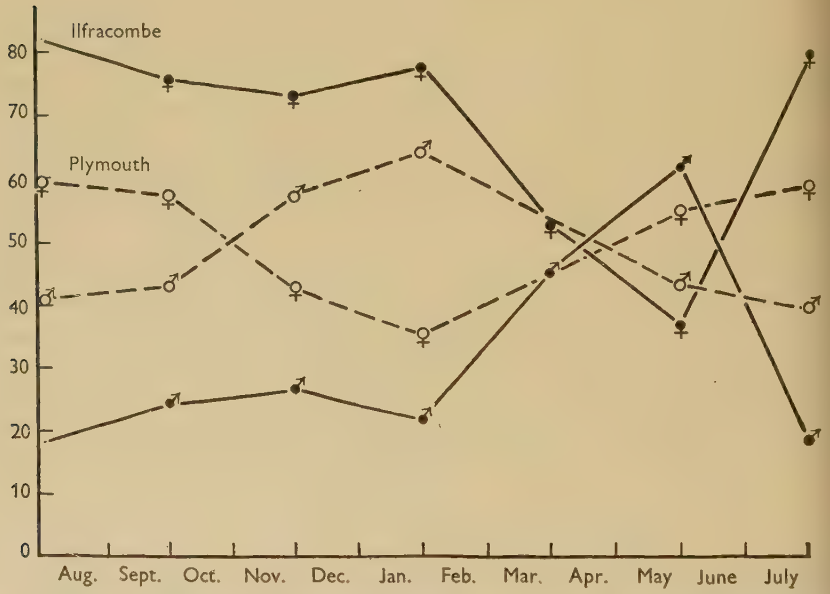
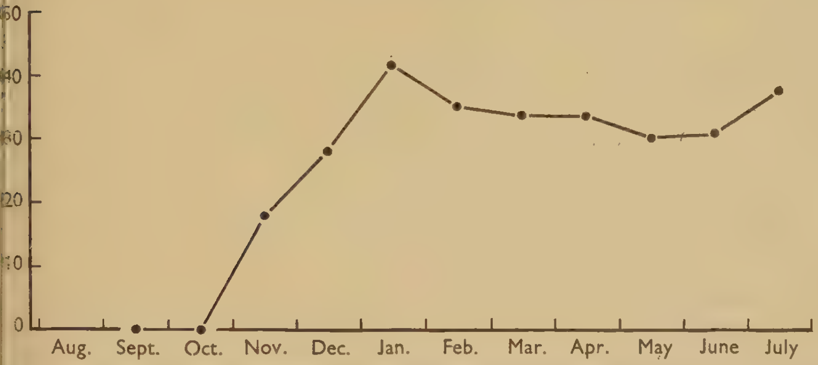


Fig. 1. *S. canicula*. The percentage of males and females in the Ilfracombe and Plymout samples at different times of year (Plymouth figures from Ford, 1921).

In September and October, however, no egg-purses were found in ninety-fo female fish. These are also the 2 months showing the smallest percentage egg-bearing females in Ford's analysis (his figures for August are based only five females of which two carried egg-purses). This observation wou accord with the statement of Lloyd (1942) that, in the few specimens *liorhinus canicula* taken at Weston in October and November, the gonads e developing.

he constant proportion of females carrying egg-purses over 8 months haps 9) of the year, and the fact that no appreciable size variation has n associated with this condition, tempts one to suggest that within this iod *all* the females in our Ilfracombe samples have been mature. Since which have just laid and which have not yet formed a hardened egg case und the next egg in the series would, on this assumption, constitute the aining two-thirds of the female population, the figures suggest that the



2. *S. canicula.* The percentage of females carrying fully formed eggs. The proportion ndicated therefore excludes (*a*) all fish which have just laid their eggs and have not yet completed the secretion of the next egg cases in the sequence, as well as (*b*) the immature females in the population.

e occupied by the fully formed egg-purse in the oviduct is one-third of the l time needed from ovulation to laying. If, on the other hand, a sub- ntial number of immature females existed in the population during the ter months, the proportion of egg-bearing fish would be expected to rise the breeding season progresses—which is apparently not true after vember.

The Sex Ratio

e total number of fish examined was 1898, of which 743 or 39·1 % were es. This presents an interesting difference from Ford's figures, in which of 4368 fish, 2401 or 55·0 % were males. Apart from this predominantly nale population in the Ilfracombe fish, the detailed analysis of the seasonal iation shows an almost exactly opposed trend to that at Plymouth; females dominate in the winter and spring months, and only in May and June is re an excess of males (Fig. 1).

he proportion of females in the Ilfracombe population is very high in tember, and remains high until the end of January; not until February es the proportion of males begin to increase substantially. Yet we have supposed that most, if not all, the females caught after November are full mature and producing fertilized eggs. Metten (1939) has proved that th folds of the shell-secreting portion of the nidamentary gland serve as a sper reservoir; viable sperm can be stored there for long periods. It is therefor quite possible that the females are inseminated in deeper waters before the come into the Ilfracombe region to lay their eggs. The fact that the Brist channel region is a natural spawning ground for the females is indicated b records of egg cases taken in Bridgwater Bay, Kilve and Blue Anchor (Lloy 1941, 1942).

An approximate estimate of the productivity of the female *S. canicula* ca be indirectly obtained from the present material. The maximum variation i age of embryos reared from any single batch of fish received has been abou 2 days. If all the females in the population are mature, we have seen tha one-third of the total time needed to produce each egg is spent by the full formed egg case in the oviduct; two eggs must therefore be laid every 6 day If the population contains immature females, the productivity must b proportionately greater; consequently each female must lay at least ten egg per month. Clark's (1922) observation that an isolated *Raia brachyura* lai twenty-seven eggs in 49 days is consistent with this high estimate of eg productivity in the dogfish.

Ford's (1921) figures, as well as the present ones from Ilfracombe, appea to suggest a scarcity of dogfish during the months of July and August. This not in fact true; the low figure for these months merely indicates the holida season at Ilfracombe, and according to Ford (personal communication) corresponding absence of records at Plymouth. Neither Ford nor the presen writer would claim any quantitative significance in the figures for total catc per month; Hoodless and Steven's table (Appendix, Table II) therefore ad an interesting and useful summary on this point, showing no sign of any fa off in numbers during the summer months.

Growth of the Embryo

In the course of the behaviour studies mentioned it has been necessary assign accurate growth stages to the embryos, particularly in the period u to the development of the pelvic fins and external gill filaments, by whic time the adult physiological pattern has been largely established. It fortunate that over the whole of this range the correspondence with the stag of development of *Squalus acanthias*, so fully described and figured b Scammon (1911), is almost exact. Scammon's tables have therefore been use throughout the work, and have made possible a fairly accurate picture of th rate of development. The marine aquarium at Bristol is refrigerated ar thermostatically controlled, but the refrigeration plant was out of action f a long period. During the whole time, morning and evening records of th er temperature were kept, and the curves in Fig. 3 show the time taken ttain the various stages described by Scammon at several different peratures. Where the temperature varied during development, the value n is the mean throughout the whole of the development up to the time embryo was removed for examination.

he curves are useful for those who wish to obtain specimens of known phology for embryological work, but they also offer one additional piece nformation. The curve for 15·5° C. is reasonably complete, and the few

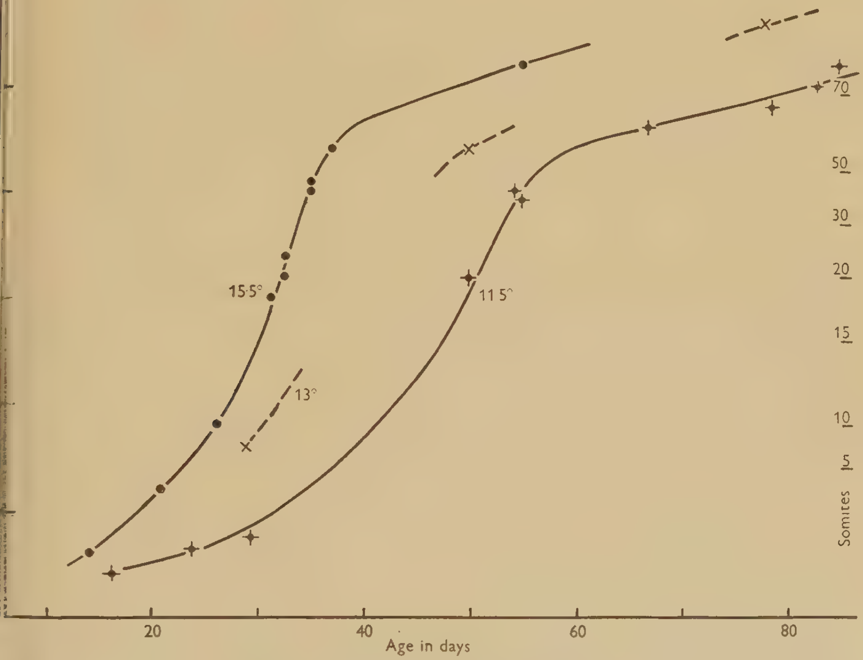


Fig. 3. *S. canicula.* Growth curves of the embryo dogfish. The stages correspond with those given in Scammon (1911).

nts available on the 11·5° C. curve correspond exactly to a development e which, stage for stage, is 1·64 times as long as at the higher temperature. what it is worth, this ratio corresponds to a temperature coefficient of of development with a value of $\mu = 20{,}000$. This figure is not very urate, since the temperatures are not known to a sufficient degree of ctness, but it is probably within ± 10 %. It appears, therefore, that the perature characteristic of the development of this elasmobranch is close he values obtained for several teleost fishes—trout, cod, plaice, etc. For mmary of these values the reader is referred to Barnes (1937).

As well as to Mr E. G. Williams of Ilfracombe, who supplied the dogfis in such excellent condition, the writer is particularly indebted to Mr C. Bee without whose accurate records and constant care of the aquarium this sho paper could not have been written.

SUMMARY

Records of nearly 2000 dogfish collected from the Ilfracombe region sugge that the spawning season of this fish starts in November and continues at lea until July. This area probably represents a spawning ground into which th females migrate during the spawning season; the males follow them muc later in April and May.

Figures are given to show the time taken at different temperatures for th embryo to develop to a series of definite morphological stages; these sugge that the temperature characteristic of the development ($\mu = 20{,}000$) is substantially the same as for several teleost species.

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Appendix

Occurrence of Scyliorhinus canicula (*L.*) *off Plymouth*

By C. A. Hoodless and G. A. Steven

From the beginning of 1949 careful records have been kept of the number adult dogfishes caught by R.V. *Sabella* while trawling off Plymouth. Th results, shown in Table II, reveal that *Scyliorhinus canicula* is present Plymouth waters all the year round, and that there is certainly no significa falling off in numbers during the summer months. Poorest catches are, fact, generally noticeable in the winter months.

is believed that the data here presented are a more accurate reflexion of ulation trends than are those of Ford (1921) and Harris; but even they are as useful for that purpose as they might be because they are affected to

TABLE II. TRAWLED ROUGH DOGFISH. *SABELLA*. STOCK SIZE

1949				1950			
No. of hauls	Hours trawling	No. of dogs	Average catch per hour	No. of hauls	Hours trawling	No. of dogs	Average catch per hour
5	10·6	1	0·1	19	29·4	5	0·2
11	21·1	8	0·4	3	5·8	51	8·8
9	14·0	3	0·2	17	23·5	28	1·2
15	25·2	44	1·7	1	2·0	3	1·5
9	15·0	195	13·0	7	12·0	86	7·2
11	17·7	188	10·6	7	11·2	70	6·2
4	5·4	52	9·6	15	27·1	49	1·8
9	13·7	95	7·0	13	21·9	78	3·5
15	23·3	162	7·0	20	34·2	114	3·3
9	12·4	104	8·4	15	23·3	168	7·2
17	29·8	83	2·8	16	26·6	127	4·8
20	35·2	102	2·9	9	16·6	32	1·9

	1951			
	No. of hauls	Hours trawling	No. of dogs	Average catch per hour
Jan.	13	22·9	70	3·0
Feb.	13	27·7	22	0·8
Mar.	3	5·9	6	1·0
Apr.	16	26·5	73	2·8
May	20	32·8	428	13·0
June	10	17·7	301	17·0
July	19	27·0	677	25·1
Aug.	9	12·4	161	13·0
Sept.	13	18·5	227	12·3
Oct.	16	21·6	418	19·3
Nov.	16	24·6	251	10·2
Dec.	10	15·1	214	14·2

ne extent by the requirements of the laboratory supply department. When nands for dogfish are heavy *Sabella* spends extra time on those grounds n which the best catches are to be expected. This influence on *Sabella*'s ches must not be overlooked, even though it cannot be evaluated.

MUSCLE RECEPTOR ORGANS IN THE PAGURIDAE

By J. S. Alexandrowicz

From the Plymouth Laboratory

(Plate I and Text-figs. 1–2)

MATERIAL AND METHODS

e occurrence of muscle receptor organs in stomatopods and decapods being
ablished, it seemed worth while to extend investigation to the hermit crabs
guridae), the more so as the thinness of the dorsal wall of the abdomen in
se animals offered favourable conditions for examination of the nerve
ments in this region. The first observations made in 1939 at Naples on
urus striatus revealed in its abdominal segments the presence of nerve cells
nected with muscles which undoubtedly represented similar receptor
ans to those found in other crustaceans. When, however, I tried later to
estigate these organs in *Eupagurus bernhardus*, the commonest hermit-crab
Plymouth waters, I was unsuccessful in obtaining suitable preparations.
agurus prideauxi gave better results, but these, too, were inferior to those
en previously by *Pagurus*. With a fresh opportunity to work at Naples in
umn 1950, I resumed the investigations with *P. striatus* and also with
calidus which proved to be very suitable for this purpose. The present
ount is based chiefly on these two species of the subfamily Pagurinae.
servations on *Eupagurus prideauxi* have shown that their muscle receptor
ans, apart from some unimportant differences in their topography, are
de on the same pattern and therefore the description given holds good for
two subfamilies of the Paguridae.

he nervous elements were stained by the same methylene-blue technique
cribed previously (Alexandrowicz, 1951[1]). Either the dye was injected
o the body of the animal or the tissue was submerged in a solution of the
, or both. The abdomen was cut along the middle line of the ventral wall
pinned to a paraffin plate with the inside turned upwards. The small
segment was not dissected and left out of consideration. All the organs
uated in the abdominal sac were removed, and the surface of the muscles
aned from the attached strands of tissue. The preparations were then put
o the methylene-blue solution. The methods of fixation and further pro-
dure were the same as set down in the paper quoted.

---

[1] Muscle receptor organs in the abdomen of *Homarus vulgaris* and *Palinurus vulgaris*,
art. Journ. Micr. Sci., Vol. 92, p. 163.

Observations

Topography of the Dorsal Muscles in the Abdomen

Text-fig. 1 A shows the dorsal wall and parts of the lateral walls of the abdomen of *Pagurus* seen from the inside. Of the 1st segment only the dorsa muscles are seen: they are composed of three portions each having a slightl different obliquity of its fibres. Of the following four segments (2nd to 5th the dorsal body-wall and parts of the lateral walls are represented.

The dorsal portion has a segmental arrangement of the muscles and i distinctly delimited from both sides by a gap in the muscle layer. The dorsa (extensor) muscles consist of parallel longitudinal fibres, the length of whic corresponds with the length of the respective segments. The fibres of th successive segments meet end to end and become attached along the line marking the limits between the segments.

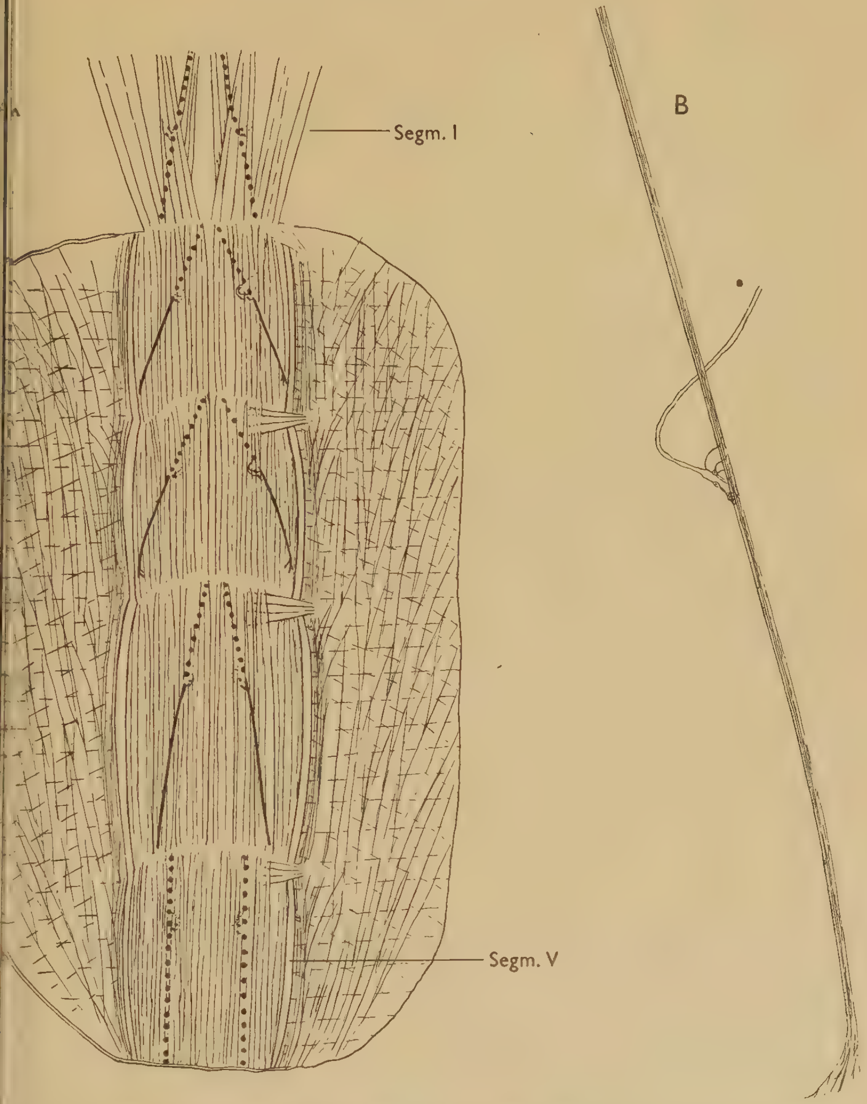
Near the anterior edges of the segments there are small transverse muscle situated asymmetrically on the left side. Their fibres converge towards th basis of each pleopod.

In the lateral walls the muscles are set in three layers. Those of the outer layer ar oblique, those in the middle transverse, while the fibres of the inner layer have a mor complicated disposition. They are chiefly longitudinal, forming stronger bundles alon the gap separating them from the dorsal muscles; more laterally, both longitudinal an oblique fibres are present, anastomosing with each other. Since the oblique fibres o this inner layer intercross the direction of those of the outer layer, there are place where fibres running in four different directions can be seen.

The median longitudinal bundles of the inner layer, being interrupted on the lef side by the transverse muscles of the pleopods, show a segmental arrangement: al other muscles of the lateral wall do not exhibit any segmentation.

Muscle Receptor Organs

As in *Homarus* and *Palinurus*, the two species of Macrura investigate (*loc. cit.*), there are in the Paguridae two pairs of muscle receptor organs i each of the 1st to 5th abdominal segments. They are each composed of a ver thin but long muscle and a nerve cell which connects with the muscle b means of dendritic processes and sends its axon towards the central nervou system (Text-fig. 1 B; Pl. I, fig. 1). The two muscle receptors situated on th same side, which must be regarded as individual units, each have particula features of their own. Their cells are of two different types, one having ver long and the other very short distal processes. In the further description th cell of the first type will be referred to as cell 1 and that of the second type a cell 2, and the muscles connected with these cells as RM 1 and RM 2 respectively. For designation of the muscle receptor organ as a whole the abbreviation MRO will be used.



-fig. 1. A, view of the dorsal wall and parts of the lateral walls of the 1st to 5th abdominal segments in *Pagurus* showing the position of the muscle receptor organs in relation to the dorsal muscles. The two MRO on each side are represented by a single line; the dotted line shows the MRO or their parts which, seen from inside, are covered by the fibres of the dorsal muscles. B, two muscle receptor organs of the 2nd segment showing their relative length and thickness, drawn to scale. The axons of the receptor cells viewed from inside appear as passing under the muscles; actually they cross these muscles on their dorsal side.

Muscle Components of the MRO

The muscles of the receptor organs have a length equal to or even slight greater than the length of the segments, but are very thin having a diamet only about 30–40 μ each, the RM 1 appearing to be somewhat thicker. Th run close together, and at first sight might be taken for a single fibre (Tex fig. 1 B). Their position in relation to the dorsal muscles is represented Text-fig. 1 A. The dotted lines indicate the receptor muscles or their portio which, seen from inside, are covered by the fibres of the dorsal muscles.

In the 1st abdominal segment the receptor muscles run near the outer edg of the median portion of the dorsal muscle, and following the course of i fibres have a slightly oblique direction. In this segment the RM lie near the dorsal surface of the muscles and thus in the preparations stretched wi the ventral surface uppermost do not stain as readily as in the following thr segments. To observe them, therefore, it is advisable to attach the preparatio dorsal side uppermost.

In the following three segments (2nd to 4th) the RM have a nearly identic course. Their anterior attachments are at the edges of the respective segmen near the median line of the body, and here the RM lie dorsally, i.e. upon tl fibres of the extensor muscles. From these points of insertion they ru obliquely backwards and outwards, at the same time passing gradual through the layer of the extensor muscles from their dorsal to the ventr surface to emerge finally on the latter at about the middle of their cours From here they run uncovered by the muscle fibres (as observed from tl inside) up to their ends which are situated near the lateral margins of tl extensor muscles and not far from their posterior attachments. The ends the RM which sometimes split into several strands are inserted into the fasc lining the inner surface of the muscles. Their course in respect to the layer the extensor muscles may be briefly defined as extending from a point whic is medial and dorsal to a point situated laterally and ventrally. It is cle from this description that the RM are completely independent of the neigl bouring muscle fibres in the course which they run, as is at first sight appare from their oblique directions. Owing to this obliquity the RM have a great length than the fibres of the extensor muscles.

The posterior attachments of the RM in the 4th segment are situated a litt nearer the median line than in the 2nd and 3rd segments and as, moreove the 4th segment is much longer than the others, its RM have a less obliq course.

In the 5th segment the RM anteriorly have superficial attachments simil to those in the foregoing. Running backwards they pass deeper into the lay of the dorsal muscles but do not emerge on their ventral surface, endi between the dorsal muscles at the edge of the segment. In all their cour they remain nearly parallel to the median line. Owing to their deeper setti they usually stain less distinctly or even remain invisible.

he arrangement of the RM in *Pagurus calidus* is the same as in *P. striatus*.
upagurus prideauxi the RM have a less oblique direction and run a little
ner from the ventral surface. Their still deeper setting in the muscle layer
resumably responsible for the failure in getting satisfactory staining in
ernhardus. In the 1st abdominal segment of that species, where the dye
have access from the dorsal side to the superficially situated MRO
stain quite well.

is easy to recognize the two RM as individual muscle units owing to their
arkable property of staining differentially in methylene blue. As a rule
of them, and this is nearly always RM 1, remains almost colourless, while
other stains blue or even dark blue (Pl. I, figs. 1, 5). Rarely the reverse
tion is observed and also rarely both RM appear stained in the same or
ly the same hue. Thanks to this difference in colour it may be stated that
1 is situated laterally and somewhat dorsally to RM 2. The differences in
appearance of the two muscles might not, of course, be noticeable when
action of the methylene blue was not long enough or was impeded—as
instance in the anterior portion of the RM situated within the muscle
r—and not easily reached by the dye. For that reason it has been difficult
etermine whether both muscles have their anterior insertions at exactly
same point or, as was found in *Homarus*, at different points. It may,
ever, be assumed that if there is separation between these points of
hment it cannot be great.

he receptor muscles have a similar structure as in the Macrura, i.e. are
posed of bundles of myofibrils and of connective tissue fibres running
itudinally between them. The connective fibres are more abundant in
1 than in RM 2.[1]

ve Cells

he nerve cells are situated at about the middle of the length of the
ptor muscles, rather a little anterior to the mid-point, and consequently
lie near the place at which the MRO appear on the ventral surface of
muscle layer; hence in the preparations with this surface uppermost
nerve cells can be well exposed for observation. It happens however,
uncommonly, that they are covered by some muscle fibres, to remove
ch an attempt should be made cautiously.

the first segment the nerve cells are situated on the lateral side of their
cles, whereas in the 2nd to 5th segments they lie on the median side.
2 lies behind, and usually close to, cell 1, but the two cells may sometimes

---

[1] s in previous papers, I prefer to avoid the term 'muscle fibres' when describing the
onents of the MRO. Being thread-like, they are fibres in the general meaning of this
, but they are not 'muscle fibres' in a strict histological sense, i.e. elements of striated
ular tissue consisting of myofibrils embedded in sarcoplasm with many nuclei and
sed in a sarcolemma-tube.

be well separated. They differ greatly in their appearance, in the distributio
of their processes, and even in their staining properties.

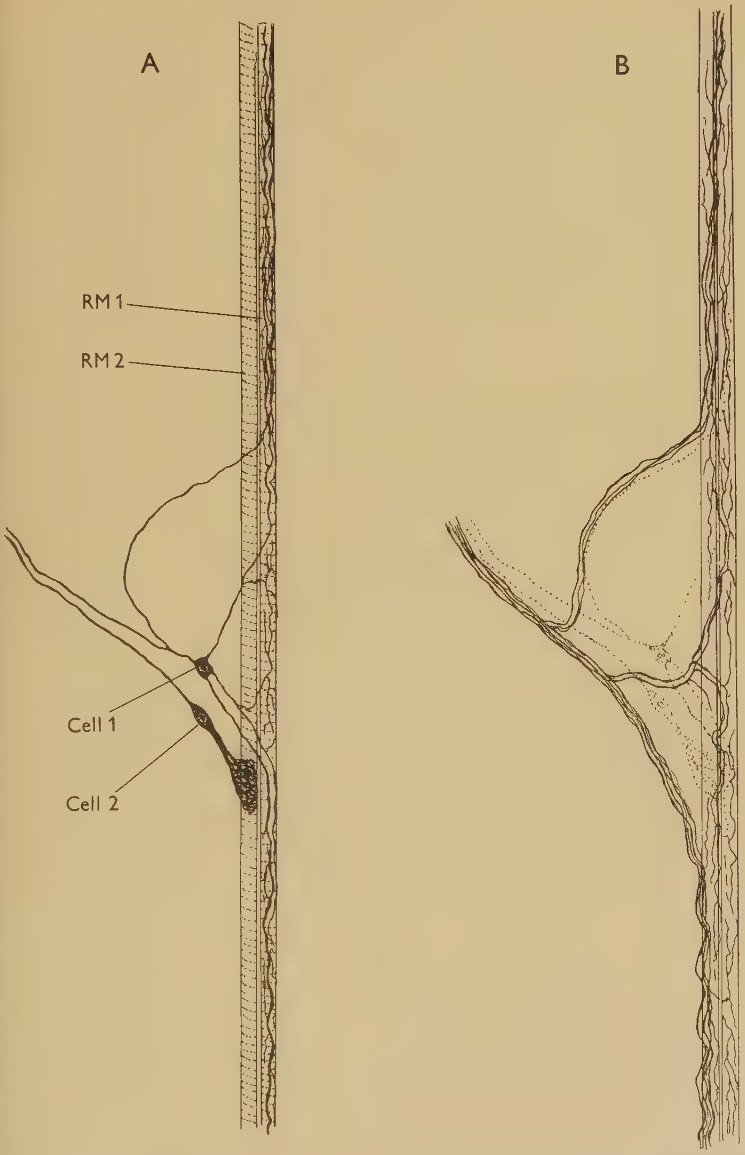
Cell 1 is multipolar, and with its processes arising from various points
the body can have a manifold shape (Pl. I, figs. 1–5). The processes, one
two of which may arise from the axon, pass on to the muscle RM 1 and ru
in opposite directions giving off numerous ramifications (Text-fig. 2 A). The
look quite like the fibres of nerves coming to the receptors from the centr
nervous system (Pl. I, figs. 3, 4). When associated with the latter in a commo
bundle the cell processes can be identified only if it is possible to trace the
up to the cell and thus detect their origin. This makes it difficult to determin
their whole course, but they are seen running far in both directions. As in n
instance can the limit of their expansion be noticed, it seems not improbab
that they might extend along the whole length of the muscle. The terminatio
of the longer branches from among those of other fibres are indistinguishabl
Those of the branches ending nearer the cell and whose origin is unquestion
able are seen to give fine filaments spreading in the RM 1. Since in all th
MRO examined in the Macrura the ramifications of the cell processes en
not on the muscle itself but on the connective tissue, it is probable that in th
Paguridae too the terminations of the cell processes are situated on the con
nective tissue fibres, which, as mentioned before, are abundant in RM
Unfortunately, it is practically impossible to discern the relations of particul
nerve filaments, as in the thin receptor muscle all the elements, viz. myofibr
bundles, connective tissue fibres and nerve fibres of various origins, run close
intermingled.

Cell 2 is as a rule bipolar with a thick distal process which gives off ve
short branches. These ramify abundantly and penetrate into RM formin
a sort of tuft which occupies the whole thickness of the muscle, but on
a small portion of its length, about three times as large as the diameter of th
muscle (Text-fig. 2 A, Pl. I, figs. 1, 3). The tuft of nerve branches is so den
that when it is well stained the muscle appears as if it were completely inte
rupted at this place by the nerve fibres. There is there, of course, connecti
tissue too.

On the periphery of the cell 2 small blue staining granules can often
observed. Possibly they are disintegrated fine nerve fibres surrounding t
cell body.

The axons of the cells join branches of motor nerves running near by.
the first segment the axons are directed laterally. In the 2nd to 5th segmen
they run at first medially then curve dorsally and laterally, thus crossing t
receptor muscles (Text-fig. 1 B). The nerve branch into which they pass ne
their origin is one of the motor branches supplying the median portion of t
extensor muscles.

Both nerve cells are protected by connective tissue arranged in concent
layers. When the cells shrink a little it may be seen that there is a sort



-fig. 2. Semi-diagrammatic view of muscle receptor organs showing in A the nerve cells with their processes and in B the nerve-fibres of central origin supplying the MRO. In both drawings the parts lying anteriorly in the animal body are shown at the top of the figures.

capsule around them but it does not leave such a wide space around the cells as in *Homarus*.

There is a striking difference in the reaction of the two cells to methylene blue. Cell 1 always stains readily when properly exposed to the action of this dye. If it does not, as usually happens in the 5th segment and not uncommonly in the others also, it is because it is covered by the muscle fibres. Cell 2, on the contrary, stains with great difficulty and no similar reason can be found for this behaviour. In many preparations only faint outlines of this cell are recognizable or even nothing at all reveals its presence—only a slight enlargement of the RM may show the area of the distribution of the cell dendrites which remain invisible (Pl. I, fig. 2*c*).

Nerves

The muscle receptor organs are richly supplied by nerves coming from the central nervous system (Text-fig. 2B). Considering the small calibre of the muscles, the thickness of the nerve bundle and its fibres running towards the MRO appear quite disproportionately large (Pl. I, fig. 5). Reaching the muscles the nerves send to them branches running in opposite directions and dividing into numerous finer fibres which penetrate the muscles through their whole thickness. Here, too, the differences in the staining of the two RM are very remarkable. In many preparations in which RM1 shows such an abundance of nerve fibres that it seems to have more nerves than any other tissue, not a single nerve fibre may be seen in RM2. In fact, they are there in great number too, but they stain very rarely. For this reason it has no been possible to find out whether the two MRO have an identical innervation or whether some of the fibres are destined for one of them only.

Having established that in *Homarus* and *Palinurus* the nerves supplying the MRO are of three different sorts, viz. motor nerves and the two accessory nerves, I expected to find a similar pattern of innervation in the Paguridae However, in these animals no such characteristic features in the appearance and distribution of these nerves could be found which would permit thei classification. In certain preparations there can be seen that the fibre approaching the MRO run parallel to each other and divide at the same spot thus behaving as do the two accessory nerves in *Homarus*. They do not however, show such differences of their diameters as the thick and thi accessory nerves in the Macrura, and their further course being uncertain n definite conclusions as to their nature can be drawn.

Comparison of the Paguridae with the Macrura

As has been pointed out before, the MRO in the abdomen of th Paguridae are made up on the same lines as in the Macrura but there are som differences. For instance, the muscles of the same pair in the Macrura hav as a rule their attachments at a certain distance from one another, can hav ore or less different course, and can differ greatly in their length. In the uridae they run close together and appear to be of equal or nearly al lengths. The dissimilarities in their histological structure are much less inct than in the Macrura, and only the staining properties indicate that are not of the same kind. On the other hand, in the nerve cells of the uridae the characteristic features distinguishing the two types of receptors, the length of the distal cell processes, are accentuated to the highest ree. In fact, the processes of cell 1 extending along the muscle attain sual dimensions. In the Macrura these processes expand on a limited which is merely a little larger than that of cell 2.

should be noted that in certain thoracic muscles in *Homarus* and *Pali-us* there are nerve cells which resemble somewhat cell 1 in *Pagurus* in that give off long processes ending between the muscle fibres. However, se cells (termed 'cells N') do not belong to the same category of receptors those situated in the dorsal muscles, for they have not their own receptor scle and otherwise differ in appearance from the former.[1]

Comparing the nerve supply of MRO in both groups it may be briefly ed that in the Paguridae there are even more than enough fibres for carrying the MRO the same elements of various sorts as found in the Macrura, but the disposition of these elements has not been discerned.

n the above account, the MRO in the thoracic segments, as described in Macrura, has not been taken into consideration although they are certainly ent in the Paguridae too. I have seen them in all the four species estigated. One of these MRO, situated superficially on the dorsal side of thoracico-abdominal muscle, is quite easily noticeable. Since in the thorax he Macrura four MRO have been found on each side it seemed very bable that the Paguridae should have the same number. However, owing echnical difficulties, I have thus far been unable to find their position or to vince myself that some of these elements are missing in these animals.

nction

When describing the MRO in the Macrura I ventured some suggestions ut their function, assuming that they are connected with the action of the tem of giant fibres and may perhaps be inhibitory to the latter during the ping movements of the abdomen. As the MRO in the Paguridae look so those of the Macrura, it is hardly probable that they would have a different ction, but since the abdomen of hermit-crabs does not perform flipping vements the question arises, if the above hypothesis should hold good, what eed may be the reason for existence of the MRO in these animals. The blem is even more complicated, since an explanation has to be found why two MRO are so different from each other.

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[1] The paper on the receptor elements in the thoracic muscles of *Homarus* and *Palinurus* is ng published in *Quart. Journ. Micr. Sci.*

I wish to express my thanks to Prof. Dr R. Dohrn, Director of the Zoological Station, Naples, for his kindness and hospitality and to the British Association for the Advancement of Science for the use of its Table.

I am indebted to Mr G. M. Spooner for his kind help in preparing the manuscript.

SUMMARY

In the dorsal wall of the abdomen in the Paguridae muscle receptor organs have been found similar to those in the Macrura. In each of the first to fifth abdominal segments there are two receptor units on each side. A receptor unit consists of a long thread-like muscle, and a nerve cell connected with this muscle and sending its axon towards the central nervous system. The muscles of each pair run close together in the layer of the dorsal (extensor) muscles, but are independent from the latter following a more or less different course.

The nerve cells in each pair of muscle receptor organs are of two types. One of them has several very long distal processes expanding over one of the muscles. The other cell has one stout distal process giving off numerous but very short branches forming with their subdivisions a dense tuft of fibres terminating in the second muscle. The axons of the cells associate with one of the branches of motor nerves supplying the dorsal muscles and pass along these branches into the main nerve trunks of the respective segments.

Each receptor organ is supplied by several nerve fibres coming from the central nervous system.

EXPLANATION OF PLATE I

All photomicrographs were made from preparations stained with methylene blue, fixed i ammonium molybdate and mounted in xylol-dammar.

Figs. 2–5 have been made with the same magnification. The differences in dimensions o cells represented depend on the various sizes of specimens.

The uneven outlines and irregular courses of the receptor muscles seen at some places ar due to deformations caused when isolating the muscle receptor organs from the abdomina wall.

The parts lying anteriorly in the animal body are shown at the top of the figures.

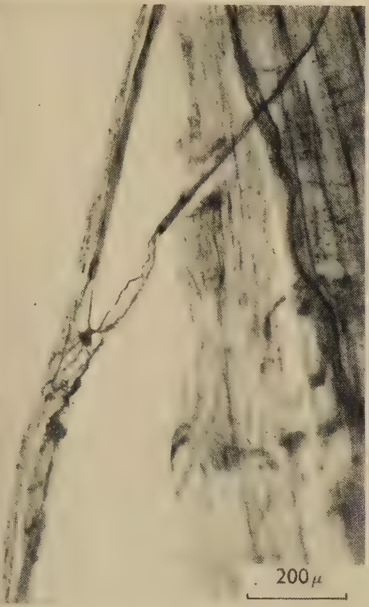
Fig. 1. *Pagurus calidus*. Middle portion of the muscle receptor organs of the 2nd abdomina segment showing their oblique course in relation to the dorsal muscles. The latter hav been partly removed. Note the position of the two nerve cells, the difference of thei shapes, the unequal staining of the two receptor muscles and the medial position of th darker stained RM 2.

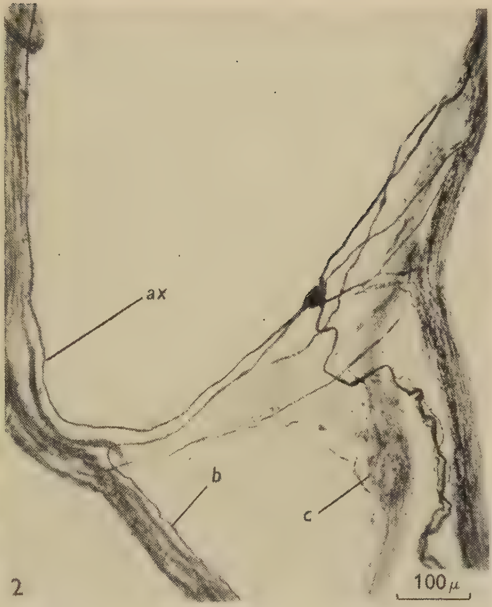
Fig. 2. *P. striatus*. Cell 1 with several processes passing on to the muscle RM 1. *ax*, its axo joining the nerve; *b*, process arising from the axon and associating with a nerve branc to reach RM 1 at a point distant from the cell; *c*, area of distribution of the processes c cell 2, the latter being invisible.

Fig. 3. *P. calidus*. Receptor organs with the cells showing the different expansions of thei processes. The unusual situation of the cells is produced artificially at fixation. *ax*, axon of both cells.

Fig. 4. *P. calidus*. Cell 1 with processes associating with the nerve-fibres of central origi

Fig. 5. *P. calidus*. Cell 1 and the bundle of nerve fibres of central origin supplying the MRC The branches of the nerves, most of them disintegrated in granules, are seen spread i RM 1, but not in the RM 2 (darker stained).





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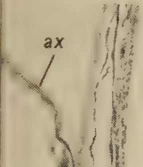

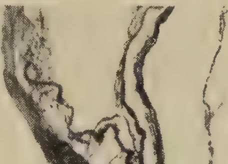

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5

HE RESPONSE MECHANISM IN ASCIDIANS

By Graham Hoyle

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(Text-figs. 1–14)

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INTRODUCTION

responses of tunicates to stimulation have been studied principally by ian (1908), Polimanti (1911), Kinoshita (1910), Hecht (1918), and Bacq 5). Jordan and Hecht established the typical responses to mechanical ulation. A light touch on the outside of either siphon rim causes that on to close, the extent of the closure being proportional to the strength he stimulus. Stronger stimulation causes closure also of the unstimulated on. This closure is accompanied by a shortening of the siphons. Stimula- of the body of the animal also causes closure of both siphons together. en the stimulation is still stronger, either applied to a single siphon or to whole animal, a violent synchronous contraction of the whole musculature rs, accompanied by the ejection of water from the branchial and atrial ties. In addition to these simple responses 'crossed responses' occur. tle stimulation of the inside of a siphon-rim results in closure of the oppo- siphon without affecting the stimulated one. Further stimulation in the e manner results in violent contraction of the whole animal with water tion through the open stimulated siphon. In some ascidians there is also flex bending of the body to vigorous mechanical stimulation. The latter activity depends on the presence of unequally thickened walls of the test and is probably due to this structural peculiarity.

Measurements of the siphon contractions during stimulation were made by Kinoshita and Polimanti, who both used *Ciona intestinalis*. They studied animals which had settled on pieces of rock, or used a clamp to grip the base of the test. Hooks were passed through the siphon rims and attached to levers writing on kymograph paper. The methods of stimulation employed were (1) mechanical, touching a siphon-rim with a glass rod; (2) electrical, using induction-coil shocks applied through platinum wires. Neither method permits very accurate control and it seems desirable to re-investigate the responses using the more accurately controlled method of stimulation employed by Pantin (1935*a–c*) for the analysis of the responses of actinians.

The studies of Kinoshita show that the responses of *Ciona* become rapidly weaker with successive bursts of stimuli. He used spring levers, thereby giving the siphons unaccustomed work to do against the lever system in addition to the normal work done against the mechanical resistance of the test. The test is very weak in *Ciona*. He claimed that the latent period of the response to an electrical stimulus was about twice that to a mechanical stimulu (0·54 sec. compared with 0·28 sec.). Polimanti, using a pulley system t transfer movements from the animal to the levers, gave the siphons less work to do. He compared the responses of both siphons simultaneously and obtained a large number of records. The results were not consistent. It i clear from his records: (1) that the heights of the contractions of the tw siphons are independent of each other on many occasions; (2) that factors which are not obviously related to the given stimuli affect the contractions In fact, the whole animal cannot be regarded as a simple mechanical system always responding to similar stimuli in the same way. Uncontrollable factors affect both the height of contraction and the rate of relaxation. Other points also appear during a survey of Polimanti's records. Contraction may not star until after several stimuli have been given, although sometimes the contraction start almost immediately after the beginning of stimulation. There are several possible explanations. The latency may be variable, the electrical stimuli ma sometimes be ineffective, or some form of inhibition may be present.

As in sea-anemones (Batham & Pantin, 1950) the responses of sea-squirt to stimulation must be observed against a background of spontaneous activity Rhythmic activity of the siphons was studied by Hecht (1918) using *Ascidi atra*, and by Yamaguchi (1931) using *Styela clava*. Both these authors ha to take special precautions to protect their experimental animals from extern vibrations by working in isolated laboratories built on either a concrete o stone floor, because ascidians are extremely sensitive to mechanical vibrations However, even with extreme precautions to protect the animals spontaneou contractions were apparent. The spontaneous activity described by Hech consisted of body-contractions and siphon closures accompanied by wat

on, at intervals of about 5 min. Yamaguchi also described spontaneous
ty with 5 min. periodicity but claimed that in filtered sea water the
rn of this activity was changed to a more regular type than that observed
dinary sea water, in which three or four small contractions alternated
larger ones.

Experimental Animals

animal principally chosen for investigation was *Phallusia mammillata*
er. Some duplicate experiments were also done on *Ascidiella aspersa*
er. These were so exactly similar in their responses that records from
species have been chosen for use in this paper to illustrate various salient
ts. These animals are not as sensitive to vibrations as *Ciona* and have
tests. *Phallusia* is especially endowed in this respect. It is available
the Plymouth Laboratory, both in estuaries, where it is exposed at very
ides, and in the Sound to considerable depths. Specimens from 13 to
m. long can be obtained without damage to the test. They survive for at
a few days in Plymouth tank sea water under circulation but do not live
when transported to London. The estuarine forms are brownish in
r owing to the presence of pigmented cells in the body-wall and the test.
deeper water forms are milky white. The test is nowhere less than a
ter of an inch in thickness except at the siphon rims. It is quite firm even
the blood is drained out and shows viscous-elastic properties when
hed. Gross alteration of the form of the test takes place fairly rapidly
the animal is removed from the sea water. The original form is recovered
turning to water if the animal is still alive. The mantle, or body-wall is
y attached at each siphon rim but elsewhere is only loosely attached to the
Contractions of the body-wall musculature result in the expulsion of
r from the branchial and atrial cavities accompanied by siphon-rim
re and withdrawal. No conspicuous movements of the whole animal
r. Hence it is possible to study siphon-rim closure as a virtually isolated
m. The musculature consists of a large number of long, narrow bands of
us relative size, distributed round the body-wall in a dense tangle in
h only a few bands stand out. The fibres anastomose considerably, and
n fine branches which are embedded in the connective tissue of the body-
. These muscles grade into those surrounding the siphons. The customary
distinction of the musculature into longitudinal and annular (Berrill,
) is hardly justified in *Phallusia* and *Ascidiella* where the siphon muscles
ar to be just an extension of the body-wall muscle complex which is
ngly concentrated in these regions so as to surround the siphons. The
ular' muscles, principally those round the siphons, have discrete,
ching ends. There are strong bands attached to the siphon rims between
appets. Contraction of these muscles and of others attached to them
gs the siphon rim inwards and downwards towards the body, the rim

being neatly folded so that complete closure results. There are few siphon encircling muscles in close proximity to the rim, but there is a strong ban with its centre about 1 cm. distant from the rim.

When the longitudinal musculature is cut near the rim on one side, tha side of the rim no longer contracts, and complete siphon closure is the impossible. Cuts in the encircling muscle band affect the contraction onl slightly. Hence it is inferred that the principal muscles concerned with siphon rim closure are those which work at right angles to the rim. The encirclin muscles control the diameter of the whole siphon cylinder, but principall serve to give the siphons rigidity and to provide attachment for other muscle

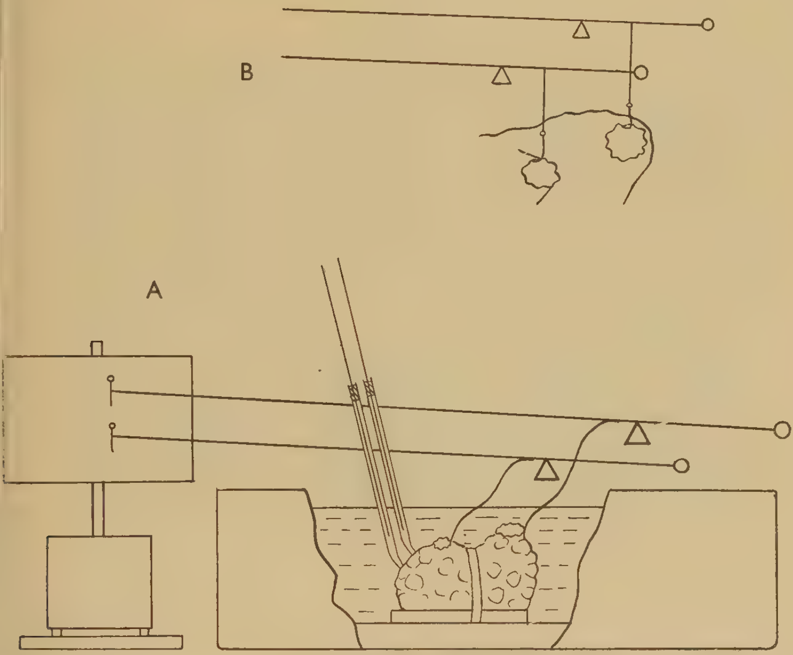
Ascidiella is found in the same estuarine habitat as *Phallusia*. Averag specimens are about 9 × 4 cm. The test is 3–6 mm. thick. Its general organization is similar to that of *Phallusia*.

The experiments to be reported involve the use of whole animals and ar therefore subject to all the difficulties encountered when trying to investigat phenomena which are undoubtedly complex and affected by a variety c factors, largely internal to the animal, which are not subject to experiment control. Nevertheless, as a preliminary to the investigation of isolated physiological units, which may prove possible in *Phallusia*, it is hoped that give adequate control, useful information may be derived from such a study.

Methods of Recording and Experimental Procedure

Animals to be investigated were allowed to rest in their natural position o a heavy glass plate, usually being secured lightly with cotton tape. The plat was placed in a large, heavy, porcelain tank or enamel basin capable of holdin several gallons of sea water. These, in turn, were supported on tables bui into a concrete floor. Kymograph motors were mounted on sponge-rubbe strips. Under these conditions the animals were sufficiently isolated fro vibrations. The recording system consisted of long levers of dried gras writing on smoked drums. The writing points were of thin glass tubing mad up to give points of the frontal type mounted at right angles to the leve (Fig. 1). Whenever the natural position of the animal permitted, a glass ar attached to the lever was allowed to touch the inside of the siphon ri thereby transmitting movements to the lever. Alternatively, a small glas hook was bent to fit over, but not penetrate, the siphon rim, and this wa connected by thread to the lever. The weight required to balance the leve when placed in the position normally occupied by the siphon rim was abo 0·4 g. so work done against the lever system was small with either metho and the animals were not unduly disturbed by either the contact arm or th hook.

Stimuli were derived from condenser discharges. They were applied throug silver/silver chloride electrodes contained in glass tubes filled with sea wate tentiometer was connected across the stimulator terminals. The frequency scharge was controlled by a metronome operating a post-office relay h also acted as stimulus marker. The electrodes were applied to the out- of the test in various positions.



ig. 1. Diagram of method of recording and manner of stimulation showing use of: (A) fixed glass arms; (B) glass hooks.

ith this method of stimulation results were, at first, extremely variable. ng the investigations it became probable that this variation is connected me way with the changing states of the animals associated with their hmicity, and with the possible effect which the shocks might have on these s. Hence in *Phallusia* various experiments have been executed in an npt to obtain an understanding of the interactions of background internal omena and external stimuli. The spontaneous activity shows a variety tterns which will be discussed in another paper. The commonest pattern, of synchronous activity of the two siphons at fairly regular intervals of min. and continuing without interruption for several hours, is illustrated ig. 2. Other types of spontaneous activity may be related to external rs (cf. Batham & Pantin, 1950). In order to be able to plan experiments h will give consistent and strictly comparable results it is important to be to distinguish two sources of variation. These are: (*a*) the variation in response to stimulation purely as a function of the stage in the spontaneou activity cycle which the animal has reached; (*b*) the response to stimulatio as a function of the previous history of the animal. It would be expected th (*a*) would show an additional variation with different test animals; (*b*) woul vary likewise but probably less than (*a*).

The first type of variation is found to have two main components. Stimu given immediately after a spontaneous contraction disturb the rhythmicit and frequently give rise to one or a series of small spontaneous contractio of variable amplitude at intervals of about ½–1 min. Kinoshita describes a 'after response' in *Ciona* which is a spontaneous incomplete contractio

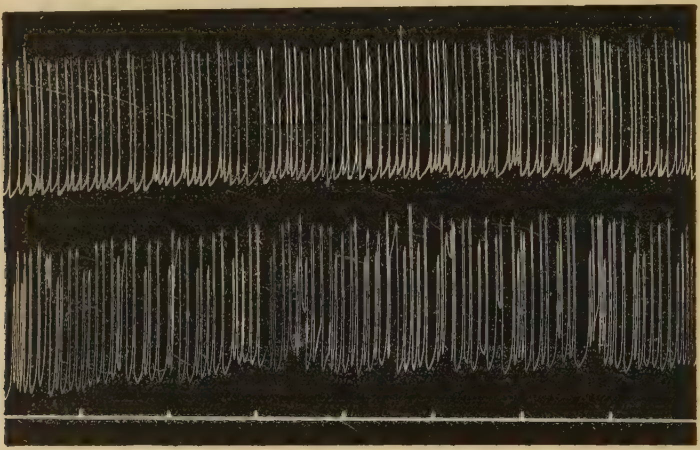
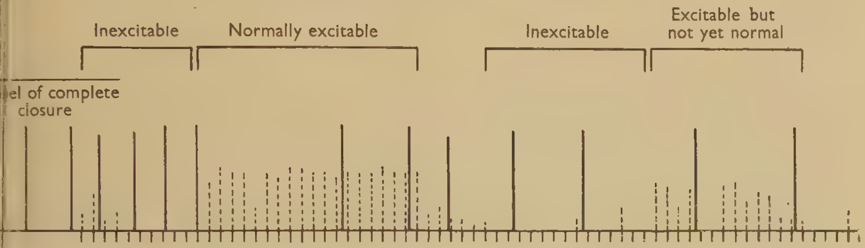


Fig. 2. Spontaneous activity of *Phallusia mammillata*. Record reads from right to left. Upper trace branchial siphon, lower trace atrial siphon. Time in hours.

occurring about half a minute after the animal has started to relax followin stimulation. It is possible that these spontaneous contractions (see Fig. 12 represent Kinoshita's 'after response'. Stimuli given just before a spontaneou contraction is due are abnormally effective in eliciting responses, for singl stimuli may elicit a squirt at this time. This is not the normal reaction t a single shock, as will be shown below. These results illustrate the extreme of the first component of type (*a*) variation which is variation during th interval between individual squirts. The second component of type (*a*) variation is a much slower variation which is revealed by giving pairs of shocks a 1 min. intervals over a period of several hours. Although the stimuli are kep at 20% above the initial threshold there are frequent phases, each lasting fo several minutes, when these stimuli give only a small response or even non at all. This observation is in line with the variations observed by Polimanti Fig. 3 shows the effect of stimulating a specimen of *Phallusia* in this way. A the start of the series illustrated the shocks were ineffective. Spontaneou activity was accelerated and then the animal entered a phase which laste

20 min. when the stimuli were equally effective. This phase ended almost harply as it began, apparently without further affecting the spontaneous vity. Later, with no increase in stimulus intensity a second period of onsiveness was apparent. Now, however, the response was weaker and re erratic. These results illustrate also the type (*b*) variation referred to. e smaller size and erratic nature of the second set of responses shows the austing effect of the earlier stimulation.

timulation experiments must, therefore, be planned to avoid coinciding n the immediate pre- and the immediate post-spontaneous contraction iod. They must also be conducted during phases of normal responsiveness, . they must not be executed too soon after a previous experiment. After nulation several minutes may elapse before the same pattern of response be re-elicited. The normal state can usually be said to be restored when the nal returns to its normal pattern of spontaneous activity.



3. The results of part of an experiment on the relation between excitability and internal phasic activity. The unbroken lines indicate spontaneous contractions, the broken lines indicate responses to electrical shocks. The marks below the baseline indicate the application of closely spaced pairs of shocks at 1 min. intervals between pairs. *Phallusia*, branchial siphon.

he experimental procedure used is therefore as follows. Only healthy cimens showing rapid movements of the siphons are used. Two equal test cks 1 sec. apart and of suitable strength are given to the animal about in. after a spontaneous contraction. If the animal gives a good response rther minute is allowed to pass and then an experiment is carried out. mediately after the experiment the animal is allowed to rest for 10–15 min., onger according to the length of the experiment, so that normal rhythmicity estored. The procedure is then repeated. In about 5% of such tests the ponse is small or, rarely, absent, and a pair of shocks does not elicit quirt (*vide* above types of variation). After a few minutes the response has ays been normal again, further indicating that occasional phases of unponsiveness are a normal feature of ascidian behaviour.

RESULTS OF EXPERIMENTS

Stimulus Strength

The electrodes, separated by about half an inch, were placed in various po tions on the test. The battery voltage and potentiometer were roughly adjust until a pair of shocks gave a response. The potentiometer was then adjust more carefully. The minimum strength required to produce a response vari with the position of the electrodes and the size of the animals. It is least wh near the siphons. The strength required is usually large in *Phallusia*, of t order of 100 V. from an 8 μF. condenser across most of a 50 K. potentiomet These facts are to be expected in view of the variable thickness of the test a the extent of short-circuiting of the shock. In *Ascidiella* the strength requir is only about one quarter of the *Phallusia* value. The magnitude of the respon to threshold stimulation does not vary with the position of the electrodes. the least effective stimulation strength every stimulus is not always effecti (Fig. 8*a*). A stimulus decrease of 0·5% makes the stimuli ineffective. Su threshold stimuli do not summate to produce a response.

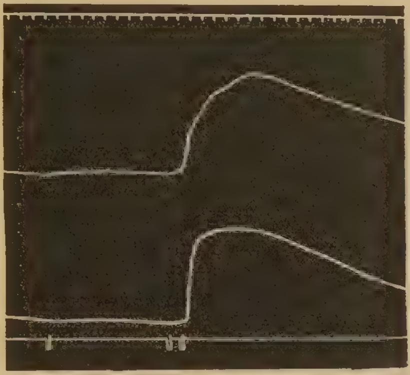
Increasing the stimulus strength above the threshold value does not aff the height of the response, the excitable system being activated in an all- nothing manner by the electrical shocks (e.g. Fig. 6). The latency of respo to a single shock is about 0·6 sec. in *Phallusia*, and there is no apprecia variation in the latency with the electrodes in different positions using t method of recording.

The excitable system

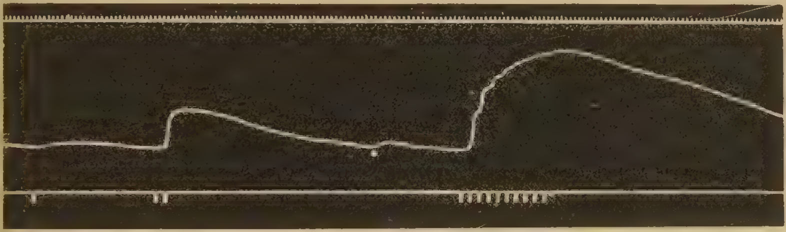
It is not possible at present to decide what the excitable system is, althou it is clear from the results of the experiments on *Phallusia* and *Ascidiella* t during electrical stimulation the muscles receive the stimuli through a rapi conducting system which is excited in an all-or-nothing manner by t stimuli. Von Buddenbrock (1928) stated that in ascidians there is a nerve n work in the body-wall, but this statement has since been strongly contest by Fedele (1937*a*). Das (1936) has described nervous elements in the test *Herdmania*. It is possible that local stimulation of the test is brought ab by these elements. Whatever the excitable system may be, the situation remarkably similar to that found in coelenterates, especially actinians, wh these are stimulated in the same way (cf. Pantin, 1935*a–c*, and Bullock, 194

Facilitation

Many results are, nevertheless, quite different from those given by s anemones. A single stimulus produces a measurable response, although th is small (Fig. 4) and occasionally ineffective (Fig. 8*b*). The second of a pair shocks at intervals of 1 sec. down to about 0·3 sec. (the smallest interval use produces a large, fast response (Figs. 4–6). Quantitative measures of the si of this response are not of great value, but it may be stated that over this ran terval between shocks there is no significant variation in the magnitude e response (Fig. 7*a*, *b*). When the first stimulus is ineffective the second



ig. 4. Responses to a single shock and to a pair of shocks at 1 sec. interval. *Phallusia.* Upper record atrial siphon, lower record branchial siphon.



. Responses to a single shock, to a pair of shocks and to a consecutive series of ten shocks. *Ascidiella.* Branchial siphon. Time in sec.

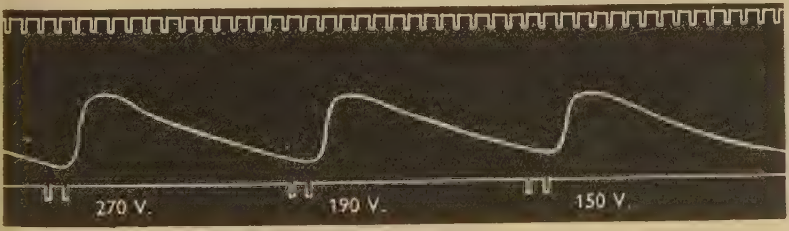


Fig. 6. Responses to pairs of stimuli at various voltages. Threshold was 145 V. *Phallusia.* Branchial siphon. Time in sec.

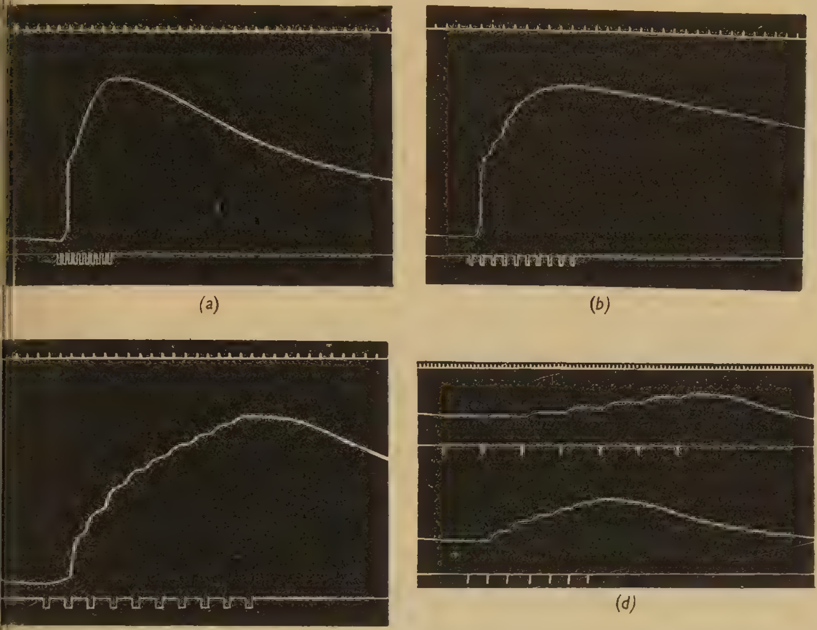
act like a normal first and the large response is then given to the third . 8*b*). Occasionally, three or even four stimuli are necessary before a first ctive stimulus appears.

The second of a pair of shocks in rapid succession produces a respon which is so much larger than the response to the first that it looks as thoug a different effector mechanism has been brought into play. Lengthening t interval between a pair of shocks shows, however, that this large response simply due to an extensive facilitation consequent upon the arrival of a fir effective stimulus. At frequencies of 1 per 2 sec. and lower the height of th response to a pair of stimuli is reduced (Fig. 7*c*). There is still an enhancin effect of the first stimulus at a frequency of only 1 per 10 sec. (Fig. 7*d* The state of facilitation develops rapidly, as in anemones, stays at its maximu for 1·5–2 sec. and then gradually declines, becoming negligible after 10–15 se After a closely spaced pair of shocks the aperture is almost half-closed an subsequent shocks produce contractions which serve to close it more com pletely. Further stimuli after the first two produce a typical smooth-muscl type staircase. The steepness of the staircase increases with increasin frequency of stimulation (Fig. 7*a–d*).

Functional significance

The functional significance of this type of response is fairly clear. The rap rise in response to the second stimulus of a closely spaced pair is the chara teristic squirt of the ascidians. The first stimulus simply closes the apertu slightly, the usual response to a slight mechanical vibration or light touch the siphon. The second, effected by stronger vibration or contact, results a rapid ejection of water through the closing aperture. This 'ejection refle (Jordan, 1908) has frequently been described as being of use in removin sources of irritation from the branchial cavity, ejecting sexual products faeces, etc. During spontaneous activity the animals seldom close the sipho much further than the closure produced by two or three shocks in rap succession. Complete closure only follows persistent stimulation. When t animal has recently been stimulated, or during periods of natural unr sponsiveness, electrical stimuli produce weaker responses with increas latency and slower rate of rise of contraction (Fig. 14, and cf. also Kinoshi and Polimanti). These consequences, which are apparent after only one burst electrical stimuli and increase progressively with further bursts until the anim is relaxed but inexcitable, are consistent with the view that the vigorous norm enhancement of the response to the second effective stimulus is due to an e treme development of facilitation, and that this mechanism is easily exhauste

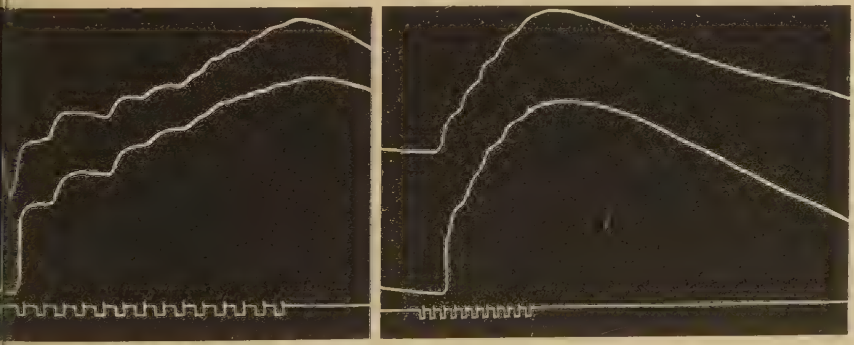
The precise way in which this exhaustion is developed must depend on t nature of the facilitation phenomenon. The indirect evidence points to t neuromuscular junction as being the site of facilitation in sea anemones. It unlikely that direct evidence will be forthcoming. In the absence of the latt it is probably unwise to speculate, but if a junction transmitter is involve exhaustion could be due either to its progressive depletion, a phenomen which is not found in any known systems, or to its accumulation which is al



(a) (b)

(c) (d)

7. Responses to: (*a*) 2 per sec.; (*b*) 1 per sec.; (*c*) 1 per 2 sec.; (*d*) 1 per 5 sec. (lower record); 1 per 10 sec. (upper record). *Phallusia.* Branchial siphon. Time in sec.



(a) (b)

3. Parallel behaviour of the atrial (upper record) and branchial (lower record) siphons showing also: (*a*) responses to just threshold stimulation at 1 per sec., not every stimulus is effective; (*b*) ineffective first stimulus with delayed response to the second and quick response to the third. *Phallusia.*

unlikely after such brief stimulation. An alternative possibility is that th phenomena are at least partly intramuscular in origin. Investigations into th properties of these peculiar muscles would be of great interest. The way i which a normal animal reacts to mechanical stimulation depends upon th intensity and the frequency of stimulation. Thus Polimanti observed tha 'rapid mechanical stimulation is more effective than slow continued pressu for the same intensity of stimulus'. The same phenomenon is observed i anemones (Kinoshita, 1911). This probably means that successive senso discharges produced by the rapid prods bring the facilitation system into pla whereas the continuous stimulus may not.

Rate of Relaxation

During spontaneous activity the shape of the relaxation curve is constan but after electrical stimulation this is not the case. There is a tendency f relaxation to occur more slowly after slow frequency stimulation but there a exceptions to this generalization. In fatigued animals the rate of relaxation extremely slow. It has not been found possible to stimulate the intact fres animal electrically in such a way that the period of contraction is maintaine for more than about five minutes. After this time the siphons relax, eve though stimulation is continued at greater intensity. The animal is itse capable of maintaining the siphons closed for much longer periods, for exampl following a period of exposure. These observations, together with othe mentioned below in connexion with ganglion extirpation, suggest that relax tion is an active process in ascidians.

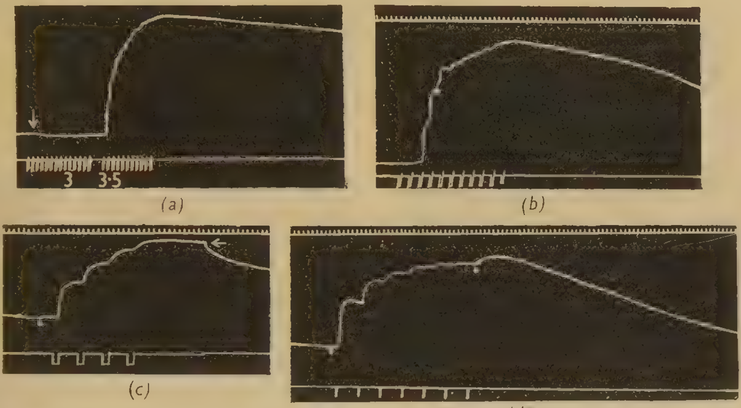
Supernumerary Responses

The appearance of supernumerary responses in the records, denotin stimulus contributions from sources other than the electrical shocks has bee noted, and white dots have been used to indicate these on the records (e. Fig. 9*c*). These are, perhaps, comparable to the supernumerary contractio described in anemones by Pantin (1935*c*). The nervous impulses responsib for these may be present in normal unstimulated sea anemones where the would not have an overt effect unless they nearly coincided in time with oth discharges, so that these together operated the facilitation system. Sin a single discharge is a recordable phenomenon in ascidians the study supernumerary contractions is there rendered more direct. When electric stimuli are applied after a small spontaneous movement it is seen that the are effective in enhancing the response to subsequent shocks (Fig. 9*c*, *a* Rhythmic supernumerary responses have also been observed, as mentione above (p. 292) (see Fig. 12), although these occur at much longer intervals tha those described in anemones. It seems highly probable that the events givin rise to supernumerary contractions are not a consequence of the electric stimuli but arise independently of them, except in the case of the rhythmic

ractions which may be due in ascidians to an effect of the shocks in
ging momentarily the frequency of spontaneous activity.

Ganglion Extirpation

xperiments have been carried out on deganglionated animals. These
nals usually respond to electrical stimulation like slightly fatigued intact
nals, but there is no great difference in the pattern of response (Fig. 11).
lan (1908) and Hecht (1918) have studied the effect of ganglion extirpation
the responses of the siphons to mechanical contact. Before extirpation
ght mechanical stimulus to the outside of a siphon rim causes a small local



(a)

(b)

(c)

(d)

9. Spontaneous movements and their consequences. *Ascidiella.* (*a*) Slight relaxation
during subthreshold stimulation; (*b*) spontaneous movement (indicated by white dot)
during stimulation experiment; (*c*) stimulus applied 2 sec. after a spontaneous movement.
Drum stopped at arrow; (*d*) stimulus applied 1 sec. after a spontaneous movement.

onse of the siphon. A larger stimulus causes closure of the other siphon
vell as closure of the stimulated one. After ganglion extirpation the
imulated siphon fails to respond, although the stimulated one continues
espond. Jordan described these responses as reflexes and regarded the
nd response as a protective reflex. If we confine the use of the term reflex
ts original usage in physiology (as 'animal spirits' ascending nerves to
ral systems and in turn descending to effect motor activity) then these
vities are better described as responses than as 'reflexes'. These responses
be studied graphically with this preparation (Fig. 10). In *Phallusia* it will
noted that in spite of the absence of the ganglion there is a slight response
he non-stimulated siphon when a single siphon is touched with a glass rod.
en the containing vessel is tapped both siphons respond together. Day
19) and Bacq (1935) agreed that the operation of ganglion extirpation
uces the general muscle tone. The fact that ganglion extirpation only

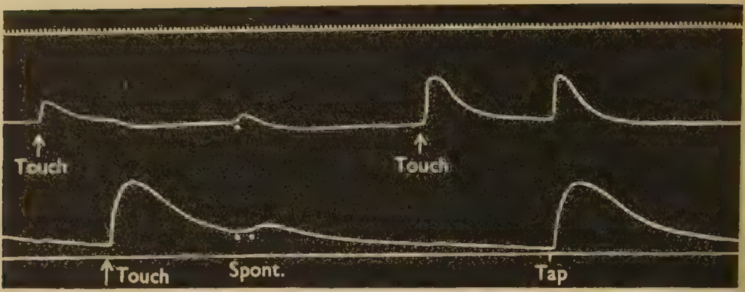


Fig. 10. Responses of a deganglionated *Phallusia* to local stimulation of the siphons and t
a tap on the tank. Note the small response of the opposite siphon. Time in sec.

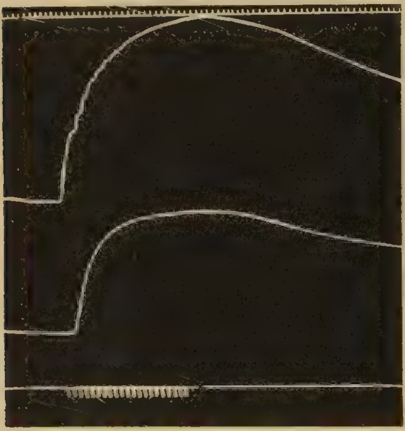


Fig. 11.

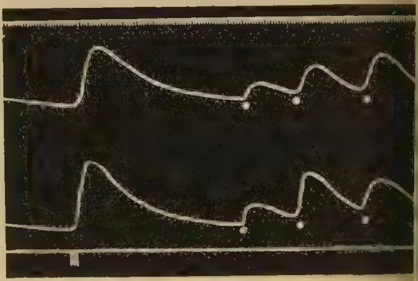


Fig. 12.

Fig. 11. Responses of a deganglionated *Phallusia* to electrical stimulation at 1 per sec. Upp
record branchial siphon, lower record astrial siphon. Time in sec.

Fig. 12. Spontaneous rhythmical contractions following an electrical stimulus (three shock
applied soon after a spontaneous squirt (not shown). *Phallusia*. Time in sec.

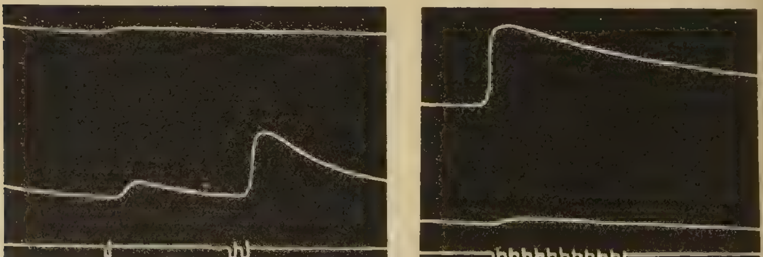
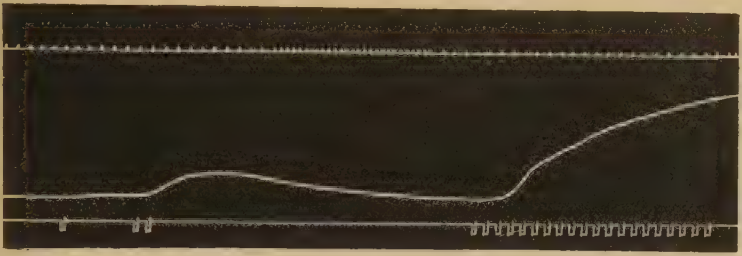


Fig. 13. Inhibition of one siphon only during stimulation experiments. Upper record atri
lower record branchial in both records. *Phallusia*. Time in sec.

s the pattern of response to electrical stimulation indirectly, by the which this operation appears to have on muscle tone, shows that entation by the ganglion is not normally involved in the squirting ments. Also, the results show that the electrical stimuli are not trans-d to the muscles by way of the ganglion.



4. Responses of *Phallusia* branchial siphon immediately following relaxation after ntense stimulation before the experiment. Time in sec. The drum was slowed down in e middle of the record to include the whole of the relaxation after the second response.

Synchrony of the Siphons

hen responses are recorded from both siphons simultaneously, it is seen hese act in unison. Water is ejected through both siphons simultaneously gh the closing apertures. The same water movements occur during aneous contractions which are also synchronous. Very rarely during lation one siphon fails to respond. In Fig. 13 the failure of one siphon n turn been followed by inhibition of the other siphon after this had d to contract. This failure of the siphons to act together has not been ved in deganglionated animals during electrical stimulation. Jordan and anti were both of the opinion that the ganglion can regulate the 'reflexes' ther inhibition or facilitation. Occasionally, small positive openings can en in the records (e.g. Fig. 9*a*) which correspond to reflexing movements e siphon rims. It is difficult to see how these movements can be brought t except by quick positive relaxation. Similar movements can be observed me of Yamaguchi's records. These observations strongly suggest that the ion can maintain or reduce tone in the muscles, and that it can prevent li from giving effective responses.

Test-free Animal

hen the animal has had its test removed it remains in a state of contrac-or several minutes and then relaxes gradually. When relaxed, spontaneous actions appear at intervals of a few minutes, as in the intact animal. e contractions involve the whole of the muscle mass simultaneously, as the eye can judge. They can also be produced by stimulating the siphon mechanically but not by touching the body-wall. In the latter case, a ripple of contraction travels outwards from the point of stimulation. Th extent of this travel depends on the strength of the stimulus. Loeb (1901) and later Fedele (1937*b*), thought that the muscles of ascidians are capable o direct excitation, and that they are also capable of propagating this excitation Bacq (1935) demonstrated slow ripples of contraction in the siphons o *Ciona*. There are certainly at least two different methods of transmission o stimuli in ascidians.

DISCUSSION

Ascidians show a number of interesting physiological peculiarities. Investigation of ascidian nerve-muscle should not only help to further the genera study of these systems, but should also throw some light on the adaptiv changes which can take place in nerve-muscle physiology in relation to a animal's way of life. The sessile sea-squirts have free-living relatives an when adequate studies have been made of both forms a comparison will b possible. There is also a parallel between the sedentary, or free-livin members of the Tunicata and the corresponding forms of the Coelenterata the sea anemones and the jellyfish. It is now possible to carry the compariso between the sedentary forms a little further.

Although no homology of intimate mechanism is postulated, and detaile consideration must await the results of further investigations, it is remarkabl that these phylogenetically unrelated groups should include sedentary representatives which have evolved independently what appears to be a simila basic method of producing a protective response from a simple muscle syste *Metridium* and *Phallusia* inhabit similar environments and are often foun together. They both feed on small particles and depend on a ciliary curre for both feeding and respiration. A large central cavity is in each concerne with these activities and there is a terminal opening into the cavity. Protectio is important not so much against predators, although these have been describe (see, for example, Yonge, 1949), as against interference with the continuity the feeding and respiratory mechanisms by foreign bodies entering therei The quick contractions of the longitudinal muscles of *Metridium* withdra the animal from the source of the stimulus and produce a small current water which helps to drive it away. Later, if the stimulus persists, the entranc to the all-important cavity is closed. In large, sedentary ascidians th facilitated quick contraction is more effective in producing a protecting jet water than the actinian system, but as in the actinian is followed later, if th stimulus persists, by further withdrawal and by closure of the aperture.

The utilization of a single effector system which has no antagonists, produce slow or quick contractions according to the nature of the stimulatio by the development of facilitation to an extremely high degree, is an efficaceo and very economical method. The systems which have been evolved by sever more highly organized animals to produce comparable effects, by the use multiple innervation, giant fibres, etc., are not necessarily more effective.

e investigations described serve to make the variations in some of the ts of previous workers, published and unpublished (personal communica-), a little clearer. Ascidians have patterns of spontaneous activity which the properties of the nerve-muscle system so that similar stimuli may different degrees of response in the same animal at different times. The rn of spontaneous activity, and other properties of the nerve-muscle m are modified for some time after a period of stimulation. The extent modification is proportional to the physiological condition of the animal he duration of stimulation.

here remain, unfortunately, large gaps in our knowledge of the pheno- on of facilitation as it appears in anemones and in ascidians. First, we do know the nature of the excitable system (this applies especially to ians) and the way in which it is activated by electrical shocks; whether, nstance, it gives trains of impulses or a single impulse for each condenser arge. Secondly, we do not know anything about the physical phenomena rring in the muscles during stimulation. There are several suggestions intramuscular factors are involved. Thirdly, we have no notion as to the nical nature of the junction transmitter. None of these problems can be d until isolated physiological units are available for investigation.

he work was done at the Plymouth Laboratory; to the Director and staff hich most grateful thanks are extended. The writer occupied the London ersity table during parts of the spring and autumn of 1951. Gratitude is expressed to Miss P. Kott and Dr J. A. C. Nicol for helpful discussion ng my stay at Plymouth, and to Prof. O. Lowenstein, Dr G. P. Wells and P. B. Medawar for carefully reading the draft and offering valuable cism.

SUMMARY

ethod is described for investigating the reactions and spontaneous activity vo species of ascidian, *Phallusia mammillata* and *Ascidiella aspersa*, which not disturb the animals unduly. The method can be used for obtaining hic records of the so-called 'reflexes' of ascidians.

due attention is paid to certain factors which affect the state of the animals, can be made to respond to electrical stimuli applied to the outside of the in a way which can be observed repeatedly in the same animal, in other nals of the same species and in at least two genera of ascidian.

lectrical stimuli applied to the test affect the excitable system of the whole nal in an all-or-nothing manner.

esponse to graded electrical stimulation reveals a facilitation mechanism ch gives enormous enhancement to the size and speed of response to the nd of a close pair of effective stimuli. It is suggested that this mechanism

is responsible for the production of the rapid squirt of the large sedenta ascidians.

A possible cause of the very rapid fatigue of ascidians to all kinds stimulation is fatigue of the facilitation mechanism following a period stimulation.

A parallel is drawn between the responses of *Metridium* and the respons of *Phallusia* and *Ascidiella*. The responses have some common features an the response mechanism appears to serve a similar purpose in the life of th animals.

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COLOGICAL AND NON-ENVIRONMENTAL ONSTITUTIONAL RESISTANCE OF THE PROTOPLASM OF MARINE ALGAE

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ome years workers on cell physiology have made a practice of investi-
g the tolerance, or, as perhaps more correctly expressed, the 'resistance',
protoplasm of plant cells to variations in the environment, both natural
artificial. Weber (1929) and Höfler (1932) have contributed valuable
s in their description of the properties of the cytoplasm of plant cells,
ing how greatly these may vary among different plants or different cells
issues of one plant. Experiments have been recorded on the resistance
rious plant cells to heat, cold, desiccation, various acids and alkalis,
ns and other chemicals, various radiations, and, for marine algae, to
ty changes in the water.

closer examination shows that these resistances are not of equal value
discernment of different types of protoplasms.

rough adaptation to the requirements of the habitat all organisms within
tain biological area show a characteristic resistance to desiccation,
sure to strong light, cold, heat, and other factors. This we shall call the
ogical resistance'. Although essentially determined by heredity, it varies
certain extent under external influences.

the other hand, there is the 'non-environmental constitutional resistance',
he resistance to substances or influences which, under natural conditions,
r become so strong that a resistance to them might play an important
in the life of the plant. This includes resistance to acids, alkalis, certain
icals, X-rays or other short waves. Such differences, often rather
ous, seem to be quite independent of environmental relations; and are
icularly suited for characterization of different types of protoplasm or of
oplasmic states. If non-environmental resistance changes during the
lopment of the plant we may interpret it, like changes in the permeability
he cell (Höfler, 1937; Hofmeister, 1938), as an indirect indication of
ange in the general condition of the protoplasm.

Ecological Resistance

ine algae of various depths from coastal regions show characteristic
rences in their resistance to desiccation, temperature, light, pH, and
r factors. The plasmolytic behaviour and osmotic resistance of some red

algae have been previously observed by Höfler (1930, 1931); the resistance t desiccation and other adverse conditions of the habitat by Baker (1909 Johnson & Skutch (1928) and others; the pH-resistance by Gail (1918 Atkins (1922*a*, *b*) and Kylin (1927); and the resistance of marine algae t cold by Kylin (1917) and others. An excellent summary of the ecology marine algae and numerous references are to be found in Fritsch (1945).

The ecological resistances for many marine algae have already bee investigated (Biebl, 1937, 1938, 1939*a*, *b*). The variations in resistance t different ecological factors establishes three definite ecological group (1) algae from the intertidal zone, (2) algae from low-water level and tid pools, and (3) sublittoral algae.

The osmotic resistance can be found by placing portions of algae in dilut and in concentrated sea water for a period of 24 hr. The osmotic value determined by plasmolysis.

The algae from the intertidal zone show an average resistance to 0·1–3 sea water (i.e. 10–300 % normal sea water), algae from the low-water lev and the tide-pools a resistance to 0·4 (0·5)–2·0 sea water, and algae from th sublittoral zone, to only 0·5 (0·6)–1·5 sea water. Such ranges in resistanc have been observed in algae from Plymouth and also from Heligoland an Naples. Table I shows the osmotic resistance of algae from various levels Naples.

The same ecological groups were found when examining the resistance t desiccation. The algae were placed, for 13 hr., in small, closely sealed ja over a saline solution of varying concentration under atmospheres of relati humidity varying from 83 to 100 %. Table II shows the results of suc experiments with algae from the rocky shores off Heligoland.

In the summer of 1951 I made some informative experiments at Plymout on the influence of direct sunlight on algae from both the intertidal an sublittoral zones.

On 21 September pieces of algae from the intertidal zone and tide-pool *Porphyra umbilicalis* (L.) Kütz. f. *laciniata* (Lightf.) J. Ag., *Cladopho utriculosa* Kütz., *Plumaria elegans* (Bonnem.) Schmitz, *Callithamnion tetr gonum* (Wither.) Ag., *Griffithsia flosculosa* (Ellis) Batt., *Phycodrys rube* (Huds.) Batt., *Sphondylothamnion multifidum* (Huds.) Näg., and from th sublittoral zone, *Dictyota dichotoma* (Huds.) Lamour., *Neomonospora ped cellata* (Sm.) G. Feldm. & Meslin, *Polyneura hilliae* (Grev.) Kylin and *Ant thamnion cruciatum* (Ag.) Näg., were placed in shallow dishes of whi porcelain filled to a depth of 1·5 cm. with sea water and exposed to dire sunlight on the roof of the laboratory for 2 hr. from 11.30 a.m. to 1.30 p. G.M.T. The daylight recorder of the laboratory showed that the light receive had been 107 kilolux-hours for that time with a maximum vertical illuminati of 61·1 kilolux and a minimum of 19·9. At the end of the experiment th water temperature was 17·8° C.

TABLE I. RESISTANCE TO DILUTE AND CONCENTRATED SEA WATER

	Osmotic resistance (sea water concentration)	Osmotic value (sea water concentration)
Intertidal zone		
adophora spinulosa Grev.	0·1–3·0	1·6
bertolonii Kütz.	0·1–3·0	1·5
hamosa Kütz.	0·1–3·0	1·6
laetevirens Kütz.	0·1–2·8	—
ngia fuscopurpurea (Dillw.) Lyngb.	0·1–3·0	—
rphyra leucosticta Thur.	0·1–3·0	—
lysiphonia pulvinata J. Ag.	0·2–3·0	2·4
Low-water level		
haetomorpha linum (Mull.) Kütz.	0·0–2·4	1·4
adophora utriculosa Kütz.	0·1–2·4	1·5
ramium berneri Schffn.	0·3–1·9	1·5
llithamnion granulatum (Wucluz.) Ag.	0·4–2·6	—
ntithamnion cruciatum (Ag.) Näg.	0·4–1·9	1·7
Sublittoral zone		
adophora prolifera (Roth) Kütz.	0·1–2·0	1·5
ramellosa Kütz.	0·5–1·7	1·6
aonia atomaria (Good. & Wood.) J. Ag.	0·8–1·5	—
riffithsia furcellata J. Ag.	0·7–1·4	1·5
flosculosa (Ellis) Batt. (=*setacea*)	0·6–1·5	1·6
schousboei Mont.	0·6–1·4	1·5
glaeothamnion scopulorum (Ag.) G. Feldm.	0·6–1·5	1·6
eramium diaphanum (Lightf.) Roth var. *strictum* Kütz.) G. Feldm.	0·4–1·4	1·4
eonosporium borrerii (Sm.) Näg.	0·7–1·5	1·5
locamium coccineum (Huds.) Lyngb.	0·7–1·4	1·7
tophyllum punctatum (Stackh.) Grev.	0·7–1·4	—
crosorium uncinatum (Turn.) Kylin	0·6–1·5	—

TABLE II. RESISTANCE TO DESICCATION

e exposed to air of the stated percentage humidity for 13 hr. The figures show the um humidity tolerated.

	Humidity (%)
Intertidal zone	
Porphyra umbilicalis (L.) Kütz. f. *laciniata* (Lightf.) J. Ag.	83·0
Rhodocharton floridulum (Dillw.) Näg.	88·0
Elachista fucicola (Vell.) Aresch.	86·0
Ulva lactuca (L.) Le Jol.	83·0
Enteromorpha linza (L.) J. Ag.	83·0
Cladophora rupestris (L.) Kütz.	83·0
C. gracilis (Griff.) Kütz.	86·0
Low-water level	
Polysiphonia nigrescens (Sm.) Grev.	86·0
Membranoptera alata (Huds.) Stackh.	94·6
Plumaria elegans (Bonnem.) Schmitz	94·6
Dictyota dichotoma (Huds.) Lamour.	94·6
Sublittoral zone	
Plocamium coccineum (Huds.) Lyngb.	98·8
Antithamnion plumula (Ellis) Thur.	98·4
Trailliella intricata Batt.	98·4
Halarachnion ligulatum (Woodw.) Kütz.	96·8

The sublittoral algae were killed completely by the irradiation; the alga from the intertidal zone and tide-pools, however, showed little or no injury Among the algae from the tide-pools only *Sphondylothamnion multifidum* an *Phycodrys rubens*, which grow in especially shaded places, were killed.

Under light of injurious intensity the necrotic cells of the red algae firs changed to a raspberry red but quickly bleached entirely under more extensiv light exposure in contrast to other lethal injuries.

If continuously exposed to two lamps of 350 W. each at a distance of 31 cm the difference in the reaction of the intertidal algae and the sublittoral alga can be readily seen after 5 days. After such a long exposure some o the algae from the intertidal zone—*Ulva lactuca* (L.) Le Jol., *Cladophor utriculosa* Kütz., *Ceramium ciliatum* (Ellis) Ducluz, *Callithamnion tetragonum* (Wither.) Ag., *Plumaria elegans* (Bonnem.) Schmitz and *Polysiphoni furcellata* (Ag.) Harv.—were bleached intensively but were still living, a shown by staining with neutral red, while the sublittoral algae and the alga from shady tide-pools—*Acrosorium uncinatum* (Turn.) Kylin, *Dictyot dichotoma* (Huds.) Lamour., *Heterosiphonia plumosa* (Ellis) Batt., *Bonnemaisonia asparagoides* (Woodw.) Ag. and *Neomonospora pedicellata* (Sm. Feldm. & Meslin—were killed.

The resistance of the protoplasm to light of high intensity therefore als shows the typical characteristics of an 'ecological resistance'.

NON-ENVIRONMENTAL CONSTITUTIONAL RESISTANCE

Experiments made in recent years have shown that the resistance of the cell of various plants to minute quantities of various elements, such as boron zinc, manganese and vanadium, differs widely (Biebl, 1947, 1949, 1950). I moss leaves or slices from the epidermis of phanerogams are brought int graded solutions of salts of the above substances we find that the death-poin of the various plants differs rather widely.

Such experiments were made with a series of solutions, of boric aci (H_3BO_3), manganese sulphate ($MnSO_4$), zinc sulphate ($ZnSO_4$), and vanadiu sulphate ($VOSO_4$), of concentrations 3, 1, 0·1, 0·01, 0·001, and 0·0001 %. Th material was immersed and examined 48 hr. later. Table III, giving the results shows the resistance of the epidermal cells of some dicotyledons and th leaflets of some mosses and liverworts to the solutions mentioned. Sulphate were chosen for the experiment, since the sulphate radicle is known to b least harmful to protoplasm.

The picture which emerges shows not that some plants are very sensitive t all these substances while others are resistant, but rather that a given plan may be relatively resistant to one substance and relatively sensitive to anothe Therefore, each separate 'combination of resistances' can be used as a differentiating characteristic for the various kinds of protoplasm.

ble III shows that the resistance of the phanerogams to boron, zinc and lium is relatively small and varies but little, while the resistance to anese differs widely.

. comparing the resistance limits of those phanerogams with that of some es and liverworts we find that the latter behave quite differently. Some es can stand H_3BO_3 or $ZnSO_4$ in solutions of as much as 3 %, and the -point in $MnSO_4$ lies generally above 20 % and in $VOSO_4$ at 5–10 %. nong the marine algae it was interesting, on the one hand, to find out her the chemical resistance of the protoplasm holds a similar exceptional ion like that of the mosses when compared with phanerogams. On the hand, the marine algae are excellent objects for deciding experimentally her there is any relationship between 'non-environmental' chemical ance and 'ecological' resistance.

E III. RESISTANCE TO BORON, ZINC, MANGANESE AND VANADIUM OF SOME DICOTYLEDONS (EPIDERMAL CELLS) AND MOSSES (LEAFLETS)

tissues were in each case exposed to the following strengths of solution: 3, 1, 0·1, 0·01 and 0·0001 %. The strengths given are of the strongest solution which the tissues d.

	H_3BO_3	$ZnSO_4$	$MnSO_4$	$VOSO_4$
eta vulgaris (mangel-wurzel)	0·1	0·01	3·0	0·01
eta vulgaris (beet-root)	0·1	0·01	3·0	0·001
rassica oleracea (kohlrabi)	0·1	0·001	0·001	0·001
rassica oleracea (cabbage)	0·1	0·001	0·001	0·001
aphanus sativus	1·0	0·001	0·001	0·001
olanum tuberosum	0·1	0·001	3·0	0·0001
aucus carota	0·1	0·001	0·01	0·001
isum sativum	0·1	±0·001	0·1	—
haseolus multiflorus	0·1	0·001	0·001	—
nium punctatum	3·0	0·1	3·0	3·0
I. rostratum	0·1	0·001	3·0	3·0
azzania trilobata	0·1	3·0	3·0	3·0
lagiochila asplenioides	3·0	0·1	3·0	3·0

gae from the intertidal zone, from tide-pools and from the low-water on the rocky shore at Wembury (east of Plymouth), and sublittoral collected at Plymouth (mostly near the Mewstone) were placed in sea- solutions of H_3BO_3, $ZnSO_4$, $MnSO_4$ and $VOSO_4$ and examined 48 hr.

four substances are soluble in sea water, but the 3 % solutions of $ZnSO_4$ $MnSO_4$ show a slight deposit at the bottom of the bottle. The experiments made with algae which, owing to the size of their cells, were especially l for microscopic examination.

ath in almost all the green and the red algae was accompanied by a strong ng of the membranes. This was most prominent in *Callithamnion onum* when placed in lethal solutions of boron and vanadium, and less placed in $ZnSO_4$ and $MnSO_4$. Moreover, in the species of *Cladophora* complex chloroplasts within the dead cells were destroyed and frequently decomposed into lumps of a dark green colour. This destructio of the normal structure of these algae was especially obvious when they we placed in strong solutions of manganese. The majority of red algae change t a red-violet colour when dead. This and the white colour of the swolle membranes, even of the small cells of *Plumaria elegans*, made dead tissu easily recognizable. In death the plastids of most red algae are displace rounded off, or occasionally stuck together. In this way also the dead cells *Dictyota dichotoma* can be distinguished from the living. In this alga th living, homogeneously coloured plastids are, as a rule, distributed close t the cell wall in the cytoplasm; in the dead cell they are displaced, stic together, and have a dark contour.

Especially difficult to recognize are the dead cells of *Ulva lactuca*, b staining with neutral red helps (6 drops of a 1 % solution of neutral red i distilled water to 50 c.c. of sea water). The living cells show red vacuole while the dead cells remain colourless. The same is true of *Porphyra*, but th cell sap of the living cells is not stained homogeneously and one can see dark re globules in the protoplasm. Similar globules are also to be found in the vacuol after neutral red staining in *Callithamnion tetragonum* and in the species *Polysiphonia*. On the other hand, *Ceramium ciliatum* shows a homogeneo red colour of the cell sap. But if a subsequent plasmolysis is made small re globules appear. This biological reaction of *Ceramium ciliatum* was of especi value for experiments in the resistance to light as described earlier in th paper, where the first injury from light was a strong bleaching of the plasti so that it was often only possible to recognize the cells as living through vit staining with neutral red. The neutral red staining of the living cells *Dictyota* is characterized by a granulated deposit. *Neomonospora* and oth red algae, on the other hand, show a diffused red staining of the cell sa In the species of *Cladophora* plasmolysis in sea water of double or treb concentration is a good test for living cells.

The results of experiments on the resistance of some algae from vario habitats to boron, zinc, manganese and vanadium are given in Table IV.

Table IV gives a clear answer to our two initial questions. (1) The resistan limits and the combination of the resistances against the chemicals used a similar to those of the phanerogams. The difference in resistance to boro zinc, manganese, and vanadium between phanerogams and marine algae is f smaller than that between phanerogams and mosses. (2) There is no relatio ship between the ecological resistance of marine algae to sea water of vario concentration, to desiccation or light exposure, on the one hand, and t chemical resistance to boron, zinc, manganese and vanadium, on the othe It differs within an ecological group, but is typical for the species.

In the above experiments the survival of the protoplasts offered an indic tion of the resistance of the cell. The death of the protoplasts indicates th the resistance limit has been exceeded, and this allows a sharp definition range of resistance. The fact that injurious influences which are not quite al affect the physiological processes within the cells can also be observed sionally by cytological methods through changes in the protoplasm osity, and through permeability or the effects of vital staining. But the mination of the internal conditions of the cell under the stress of extremely ng but not quite lethal conditions is another question. It can be better wered by examining the progress of certain physiological processes such spiration or assimilation (Montfort, 1936, 1937; Montfort & Hahn, 1950; ker & Holdheide, 1937).

BLE IV. RESISTANCE TO BORON, ZINC, MANGANESE AND VANADIUM OF ALGAE FROM VARIOUS HABITATS

he algae were placed in solutions of the same strength as in Table III, the strongest solution h they survived being recorded. Figures in brackets show that the alga was partially killed. ncentrations lower than this the tissues survived.

	H_3BO_3	$ZnSO_4$	$MnSO_4$	$VOSO_4$
Intertidal zone				
dophora ramosissima (Drap.) Kütz.	(1·0)	(0·1)	0·1	0·01
rupestris (L.) Kütz.	1·0	0·1	3·0	0·01
a lactuca (L.) Le Jol.	0·1	0·01	(1·0)	0·01
amium ciliatum (Ellis) Ducluz	0·1	0·01	(3, 1)	0·01
Low-water level and Tidepools				
triculosa Kütz.	1·0	(0·1)	(0·1)	0·01
aria elegans (Bonnem.) Schmitz	0·1	(1, 0·1)	3·0	(0·01)
lithamnion tetragonum (Wither.) C.Ag.	0·01	(1·0)	(1·0)	(0·01)
ysiphonia elongella Harv.	(1·0)	(0·1)	1·0	0·001
siphonia sp.	0·1	0·1	1·0	0·001
urcellata (Ag.) Harv.	(1·0)	(0·1)	(1·0)	0·01
Sublittoral zone				
yota dichotoma (Huds.) Lamour.	0·01	0·1	1·0	(0·01)
yopteris membranacea (Stackh.) Batt.	0·01	0·01	3·0	(0·0001)
ithamnion cruciatum (Ag.) Näg.	0·1	0·1	0·1	(0·01)
erosiphonia plumosa (Ellis) Batt.	1·0	(0·1)	(3·0)	(0·01)
monospora pedicellata (Sm.) G. Feldm. & Meslin	0·1	(0·1)	(3·0)	0·001

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SUMMARY

ine algae supply good examples of the difference between 'ecological' 'non-environmental constitutional' resistance of the protoplasm of their to variations in the environment. The resistance to ecological factors as diluted or concentrated sea water, to desiccation, or to light exposure ery similar among algae occupying the same habitat. On the basis of the

degree of resistance shown, three ecological groups can be distinguished (1) algae from the intertidal zone; (2) algae from the low-water level and tide pools; and (3) sublittoral algae.

On the other hand, there is no similarity in the resistance to chemica substances (H_3BO_3, $ZnSO_4$, $MnSO_4$, $VOSO_4$) of algae within a give ecological group. It is characteristic for a given species.

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TUDIES ON THE PARASITES OF *CARDIUM EDULE* L.: *CERCARIA FULBRIGHTI* N.SP., A *GYMNOPHALLUS* LARVA WITH A FORKED TAIL

By Robert F. Hutton

From the Plymouth Laboratory

(Plate I and Text-figs. 1–2)

xamination of 420 specimens of *Cardium edule* L., collected at Millbrook, mouth, England, revealed a species of marine cercaria differing from any r larval trematode which has been described. This parasite was present ve of the 420 specimens. The name *Cercaria fulbrighti* is given to this ies in honour of U.S. Senator J. William Fulbright of Arkansas.

his work was carried out at the Plymouth Laboratory of the Marine ogical Association while the author was holding a Fulbright Scholarship. am grateful to Dr V. Fretter and Dr B. Dawes for reading and criticizing manuscript. In addition to thanking Dr J. S. Alexandrowicz for reading offering valuable criticism for the improvement of my manuscript, I am bted to him for taking the photomicrographs.

THE TREMATODE PARASITES OF *CARDIUM EDULE* L.

le I contains a summary of the trematode parasites which have been rded from the cockle. In the table there is some doubt as to whether *aria dichotoma* Müller and *C. fissicauda* La Valette St. George are rent species: they may represent, as Lebour (1912) considered them, one; or, if two, they are closely related. Pelseneer (1906), reporting

TABLE I

Parasite	Author	Reported by	Encystment or Sporocyst
phalopsis gracilescens	(Rudolphi, 1819)	Lebour, 1912; etc.	Sporocyst
ucephalus haimeanus	Lacaze-Duthiers, 1854		
aria dichotoma	Müller (according to La Valette St. George, 1855)	Pelseneer, 1906; etc.	Sporocyst
ssicauda	La Valette St. George, 1855	Johnstone, 1904	Sporocyst?
rchis acanthus	(Nicoll, 1906)	Lebour & Elmhirst, 1922	Encystment
ercaria purpurae sp.inq.	Lebour, 1912		
asthla leptosoma	(Creplin, 1829)	Lebour, 1912?	Encystment
chinostomum secundum	Nicoll, 1906		
aria mytili sp.inq.	Lebour, 1912	Lebour, 1912	Encystment
rigata	Lebour, 1908	Rees, 1939	Sporocyst
dapedon rachion?	(Cobbold, 1858)	Lebour, 1912	Sporocyst
epodora rachiaea?	(Cobbold, 1858)		
aria margaritae sp.inq.	Lebour, 1912	Lebour, 1912	Sporocyst
ambrensis	Cole, 1938	Cole, 1938	Sporocyst

C. dichotoma from *Cardium edule* (and from several other bivalves), an believing but one species to be involved, gives the following evidence as to wh the name *Cercaria fissicauda* should be suppressed in favour of *C. dichotom* for this larval trematode.

> *C. dichotoma* a été trouvé libre, en mer, à Nice (3). C'est de cette espèce, que notr forme se rapproche le plus; Villot était aussi de cet avis dans sa communicatio préliminaire (4); mais dans son travail définitif, il donne au parasite de '*Scrobiculari tenuis*', le nom de *C. fissicauda* (5). Or *C. fissicauda* (6) en diffère bien plus et es parasite dans un Gastropode pulmoné d'eau douce: *Limnaea stagnalis*.
>
> (3) De La Valette St. George, *loc. cit.*, pl. II, fig. 1.
> (4) Villot. Sur les migrations et les métamorphoses des Trématodes. *Compte Rendus Acad. Paris*, t. LXXXI, 1875, p. 475.
> (5) Villot. *Ann. d. Sc. nat.* (*Zool.*), sér. 6, t. VIII, p. 37.
> (6) De La Valette St. George, *loc. cit.*, pl. II, fig. 6 et H.

Materials and Methods

Living material was used to study the excretory system. Measurements wer made on living specimens compressed between a glass slide and a cover-glass as well as on whole mounts fixed in sublimate, stained with Giemsa, an mounted in Canada balsam. Tissues of infected specimens of *Cardium edul* were fixed in Bouin's solution. Serial sections ($6\,\mu$) were stained wit Ehrlich's haematoxylin and eosin.

Observations

The development of *Cercaria fulbrighti* n.sp. within the sporocyst differs fro that of any other larval trematode yet recorded. In young developing cer-cariae forked tails appear (Text-figs. 1 *d–k* and 2 A, B; Pl. I, figs. 1, 4), while *full developed* cercariae (Text-figs. 1 *l* and 2 A, C; Pl. I, figs. 1, 2 and 4), althoug still within the sporocyst, never exhibit tails. Throughout the paper, the ter '*fully developed* cercariae' is used to designate cercariae, still enclosed withi the sporocyst, which have had, in the course of their development, forked tails, but which no longer possess these structures. The tail of thi larval trematode may degenerate completely while it is still within th sporocyst. The development and subsequent degeneration of the tail i illustrated in Text-fig. 1; the drawings were made with the aid of a camera lucida using the same magnification. Both tailed and tail-less forms may b present within a sporocyst at the same time, with five being the maximum number of *fully developed* cercariae. Fork-tailed cercariae, capable of active swimming (provided the tail has not started to degenerate), may be liberated from the sporocyst. However, the free-swimming tailed forms, thus fa observed, were not as well-developed as compared with cercariae whose tails had degenerated and which were still within the sporocyst. From these observations the possibility of two types of life-history arises; namely, one type in which the free-swimming or tail-less larvae enter a second interiate host, and a second type in which the tail-less larvae are transferred ctly to the final host.

f the 420 cockles collected at Millbrook, five (1·2 %) were found to con- sporocysts of this species, and in four of these cockles, sporocysts of at one other cercaria were found. There were two double (Pl. I, fig. 1) two triple infections. In addition to *C. fulbrighti*, both double infections ained '*Bucephalus haimeanus*', while '*B. haimeanus*' and *Cercaria cam- sis* were included in the two triple infections.

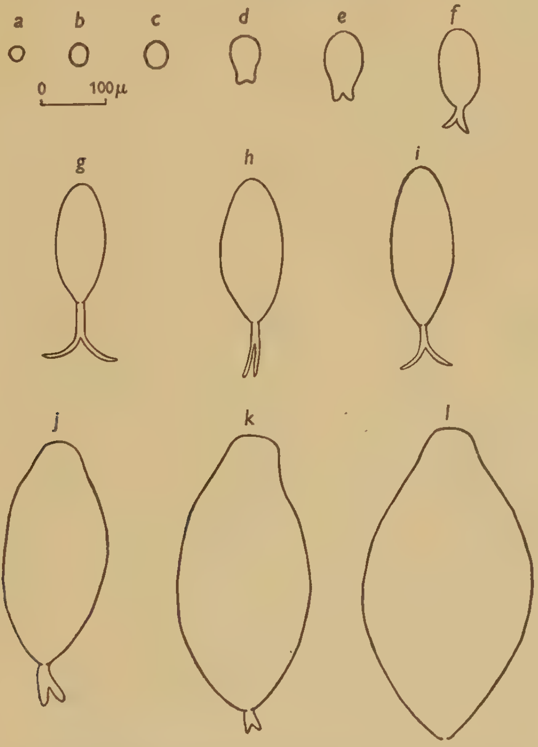


fig. 1. *Cercaria fulbrighti* n.sp.; development and subsequent degeneration of the ail which occur within the sporocyst (camera lucida drawings made at the same nagnification).

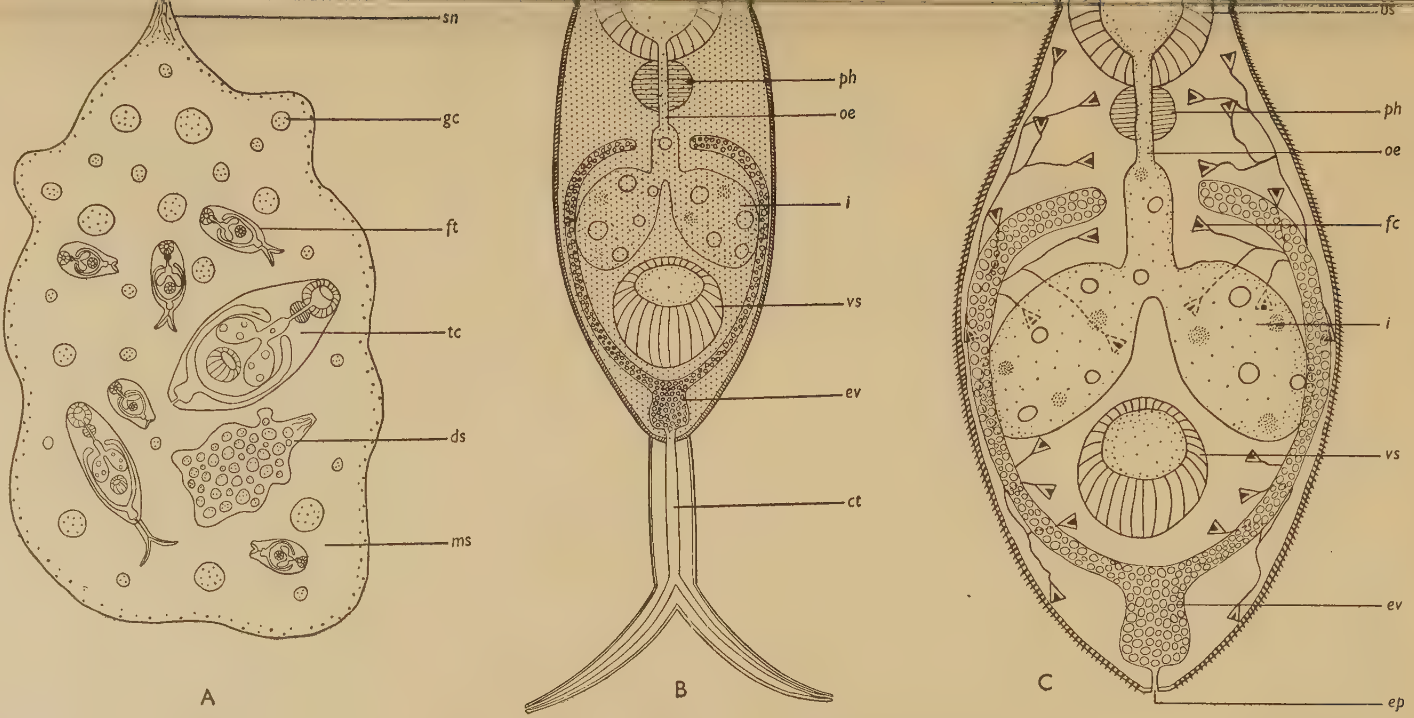
he sporocysts of *C. fulbrighti* are capable of movement by means of ıltaneously expanding and contracting parts of the body-wall. The entire y-wall, with the exception of a snout-like structure (Pl. I, fig. 1), is lved in such movements. The snout-like structure is pierced by an ing leading into the body cavity. Sporocysts of this species are very erous, but never quite so concentrated within the individual *Cardium* ose of '*Bucephalus haimeanus*' (Pl. I, fig. 1); the latter trematode, the most mon in the cockles examined, occurred in 26·4 % of the specimens.

Cercariae fulbrighti n.sp.

(Text-figs. 1 and 2; Pl. I)

Specific diagnosis. Marine, nonoculate, distome cercariae developin within irregularly ovoid sporocysts. Partially developed cercariae havin distally forked-tails (Text-figs. 1 *d–k* and 2 A, B; Pl. I, figs. 1, 4); *fully develope* cercariae tail-less (Text-figs. 1 *l* and 2 A, C; Pl. I, figs. 1, 2 and 4) while stil within the sporocyst; sporocyst liable to contain both tailed and tail-les forms at the same time. Cuticle covering the body of the fork-tailed form showing small dots arranged densely and regularly (Pl. I, figs. 2, 3 this pattern seen in sections appears to be the surface view of fine stria passing obliquely through the thickness of the cuticle; cuticle of *full developed* cercariae uniformly spined. Oral sucker slightly larger tha ventral sucker, the latter being located posterior to the centre of the body Prepharynx absent; a well-developed pharynx present leading into a wid oesophagus; intestinal caeca reaching just beyond the anterior edge of th ventral sucker. Excretory vesicle conspicuous, the lateral branches extendin forward well past the centre of the body, converging before reaching th level of the pharynx and ending a short distance from the mid-point of th oesophagus; in the *fully developed* cercaria, the terminal vesicle opening t the exterior by a short narrow duct; in the tailed form, the caudal excretor tube passing through the tail stem bifurcating at its distal end with eac branch continuing through the furcae to open to the exterior at their tips thirteen pairs of flame cells observed. Measurements based on five fixed, *full developed* specimens: body length 258–408 μ, median 328 μ; body widt 152–196 μ, median 160 μ; oral sucker width 68–82 μ, median 72 μ; ventr sucker width 62–76 μ, median 70 μ; pharynx width 20–24 μ, median 22 distance of ventral sucker from anterior end of body 124–208 μ, median 164 Measurements based on eleven living, *fully developed* specimens compresse between glass slide and cover-glass: body length 440–596 μ, median 536 body width 248–320 μ, median 288 μ; oral sucker width 100–132 μ, medi 120 μ; ventral sucker width 88–126 μ, median 104 μ; pharynx width 28–36 median 32 μ; distance of ventral sucker from anterior end of body 268–356 median 312 μ. Measurements based on three tailed specimens: body leng 100–124 μ, median 120 μ; body width 60–80 μ, median 64 μ; oral suck width 28–34 μ, median 32 μ; ventral sucker width 28–32 μ, median 30 μ; tai stem length 40–52 μ, median 44 μ; tail stem width 12–18 μ, median 16 furca length 44–80 μ, median 48 μ.

Sporocyst. Sporocysts numerous and somewhat irregularly ovoid; capable independent movement by means of projecting parts of the body-wall; a defini snout-like structure present (Text-fig. 2 A, *sn*); relatively few *fully develop* cercariae in any one sporocyst, five being the maximum number observe both cercariae and daughter sporocysts developing within the mother spor



Text-fig. 2. *Cercaria fulbrighti* n.sp. A, sporocyst containing daughter sporocyst and cercariae in various stages of development. B, cercaria before degeneration of tail begins. C, cercaria after degeneration of tail is completed. The excretory system is incompletely shown as the granules within the excretory vesicle obscured parts of some of the flame cell canals. (All figures are semi-diagrammatic.) *ct*, caudal excretory tube; *ds*, daughter sporocyst; *ep*, excretory pore; *ev*, excretory vesicle; *fc*, flame cell; *ft*, furcocercous cercaria; *gc*, germ cell; *i*, intestinal caecum; *ms*, mother sporocyst; *oe*, oesophagus; *os*, oral sucker; *ph*, pharynx; *sn*, snout; *tc*, tail-less cercaria; *vs*, ventral sucker.

cyst. Measurements based on one fixed specimen: body length 1416 μ body width 768 μ. Measurements based on one living specimen compresse between glass slide and cover-glass: body length 1250 μ; body width 880 μ

Host. *Cardium edule* L.

Habitat. Sporocysts in gonad, digestive gland, and upper part of foot.

Type locality. Millbrook, Plymouth, England.

Type material. Reference No.: 1952, 6, 26, 1/2, British Museum (Natura History), South Kensington.

Comparisons with Previously Described Cercariae

Cercaria fulbrighti differs from all previously described cercariae in that i loses its forked tail while still within the sporocyst. It was first thought tha either the fork-tailed stage (Text-fig. 2B), or the *fully developed* stage (Text fig. 2C), might have been described previously by a worker who failed to fin both forms in the sporocyst. However, a search of the literature revealed n such description: there is no larval trematode which corresponds in structura detail to either stage of *C. fulbrighti*. But, *C. dichotoma* Müller, as describe by Lebour (1908, 1912) from *Scrobicularia tenuis*, appears to be very like th tailed form of *Cercaria fulbrighti* in its proportions and in the arrangemen of the caudal excretory canals. However, because of certain morphologica differences, the author feels justified in separating these forms specificall Lebour refers to the sporocyst stage of *C. dichotoma* as 'structureless', a impression which could not be given by the sporocyst of the species describe in this paper, for it shows a well-defined snout-like projection (Text-fig. 2A and the cuticle of *Cercaria fulbrighti* shows the characteristic patter previously described (p. 320), whereas no mention of such markings i made in Lebour's description of *C. dichotoma* Müller. Lebour's figure c *C. dichotoma* shows the lateral branches of the excretory vesicle divergin laterally approximately half-way between the anterior and posterior end of the body. In *C. fulbrighti* these lateral branches extend nearly two-third of the way to the anterior end and converge almost to touch the oesophagu

Three *Gymnophallus* cercariae, in the sporocyst stage, have been reporte from *Cardium edule*; namely, *Cercaria margaritae* sp.inq. of Lebour, *C. strigat* Lebour, and *C. cambrensis* Cole. The fully developed cercaria of *C. fulbrigh* can be distinguished quite readily from each of these. *C. margarita* possesses a pair of brown eye-spots while *C. fulbrighti* does not. Also, i the former, the lateral branches of the excretory vesicle reach to the lev of the pharynx, while in *C. fulbrighti* they never extend beyond the mid-poi of the oesophagus. According to Lebour (1912), the ratio of the oral to th ventral sucker of *C. margaritae* is 2 : 3, but, as Cole (1938) pointed out, this probably a misprint for 3 : 2. In *C. fulbrighti* this ratio is approximately 9 : For *C. strigata*, Rees (1939) gives this ratio as 9 : 5. Also, she reported th

-cell formula to be $2[(2+2)+(2+2)]=16$, while in *C. fulbrighti* the umber of flame cells observed was 26. However, it should be mentioned he granules within the excretory vesicle obscured parts of the excretory n and prevented the tracing of all the canals of the flame cells. Specimens th *C. cambrensis* and *C. fulbrighti* were observed in the same cockles and re surely different species. The two species can be separated easily by ize and shape of their excretory vesicles: the lateral branches of this le of the former larva occupy most of the body and extend to the level of harynx. In the tailed *Gymnophallus*, the lateral branches of the excretory le are much smaller and never extend beyond the mid-point of the hagus. Also, the latter is much the larger and has (1) a longer hagus, and (2) larger suckers, which can be seen by the following urements comparing the sucker widths with the body length. The urements for *C. cambrensis* are Cole's (1938):

	C. cambrensis		*C. fulbrighti*			
			Tailed stage		Tail-less stage	
	(μ)	Ratio	(μ)	Ratio	(μ)	Ratio
Body length	300	*10*	120	*4*	536	*5·15*
Oral sucker width	40	*1·33*	32	*1·1*	120	*1·15*
Ventral sucker width	30	*1*	30	*1*	104	*1*

Discussion

(1909) placed those forms in which a tail is not developed in the Cerea group. He described the Furcocercous cercariae in the following s:

> tome Cercarien mit langem, an seinem freien Ende gegabeltem Schwanze, in en der schlanke Körper nicht zurückgezogen werden kann.[1] Entwickelung meist hr langgestreckten Sporocysten, welche (ob bei allen Arten?) selbständig glich sind, nur bei einer Art angeblich in Redien.
>
> [1] Vgl. hierzu auch die vorstehende *C. mirabilis*.

bour (1912), reviewing the British marine cercariae, placed the tail-less s in the *Gymnophallus* group and defined them as follows:

> rcaria tail-less, developed (in the only species in which the sporocysts are known) re or less spherical sporocysts in Pelecypoda. Oval body covered with spines. epharynx, conspicuous pharynx: short and broad intestinal caeca seldom reaching d ventral sucker. Ventral sucker always behind centre of body. Excretory vesicle forked, reaching far forward anteriorly.

e assigned the fork-tailed form (*Cercaria dichotoma* Müller) to a separate p and stated that in structure this group was closely allied with the *nophallus* group.

Sewell (1922), modifying Lühe's scheme of classification somewhat, place those forms 'in which development occurs in rediae or in simple sporocyst and in which all trace of a tail is absent' in the Cercariaea group. He revise and extended the three groups of Furcocercous cercariae created by Co (1917), placing in group 1 the apharyngeate, brevifurcous cercariae and i group 2 the pharyngeate, longifurcate cercariae (with the exception *C. indica* XXII which he included in the 'Baiswan' subgroup of group although it had certain characteristics in common with the brevifurcous ce cariae). Group 3 includes those forms which Sewell believed to hav originated from a monostome stock and in which the acetabulum was extremely small and rudimentary or completely absent.

Miller (1926), in his monograph on Furcocercous cercariae, modifie Sewell's scheme of classification and considered the presence or absence a pharynx to be of more significance than the presence or absence of a ventr sucker in determining a natural scheme of classification. He subdivided th pharyngeal cercariae into 'brevifurcate' and 'longifurcate' larvae, the forme with 'furcae usually less than one-half the tail-stem length' and the latter wit 'furcae longer than one-half the tail-stem, sometimes exceeding it'. He mad a comparison of the characteristics of the brevifurcate and the longifurcat larvae known at that time.

At one stage in its development *C. fulbrighti* has many characteristics i common with Miller's 'pharyngeal longifurcate distome cercariae', but diffe from this group in certain respects, the most notable difference being th in this cercaria the excretory openings are at the tips of the furcae while i Miller's longifurcate larvae the openings are typically mid-furcal. In a late stage, when the tail disappears and spines develop, *C. fulbrighti* has all th characteristics of Lebour's *Gymnophallus* group. These characteristics we quoted previously.

Cort (1918), discussing the tail-less cercariae, points out that the provisional *Cercariaeum*, based only on the absence of the tail in the full developed cercaria within the sporocyst or redia, is evidently an unnatur group. It may yet be assumed that members of this group, however unnatura have evolved from cercarial ancestors which possessed tails. As shown abov the forked tail of *Cercaria fulbrighti* degenerates while still within the sporocys The tail-less cercaria cannot therefore have a free-swimming stage. This much i evident, even though information is lacking on the further stages of this specie

Since *C. fulbrighti* presumably develops into a member of the genu *Gymnophallus* Odhner (1900), there appears to be sufficient evidence t suggest a phylogenetic relationship between this group and certain of th furcocercous cercariae.

As was already noted, the adult stage of *Cercaria fulbrighti* is almos certainly a species of *Gymnophallus*. It is interesting to note the remarkabl similarity between this larva and the adult stage of *G. choledochus* Odhne n Nicoll (1923) recorded from the gall-bladders of three British birds: ommon sheld duck, *Tadorna tadorna* (L.); the eider duck, *Somateria sima* (L.); and, the king eider, *S. spectabilis* (L.).

SUMMARY

mmary of the trematode parasites which have been recorded from the e is given.

furcocercous, *Gymnophallus* larva, *Cercaria fulbrighti* n.sp., from the e bivalve *Cardium edule* L., is described from Plymouth, England. The f this larva may degenerate completely while the cercaria is still within porocyst.

multaneous development of more than one species of cercaria has been ded in four out of 420 specimens of *C. edule* L. Two double and two infections were found.

ailable evidence suggests a phylogenetic relationship between the *Gymno-* *us* group and certain of the Furcocercous cercariae.

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EXPLANATION OF PLATE I

Cercaria fulbright n.sp.

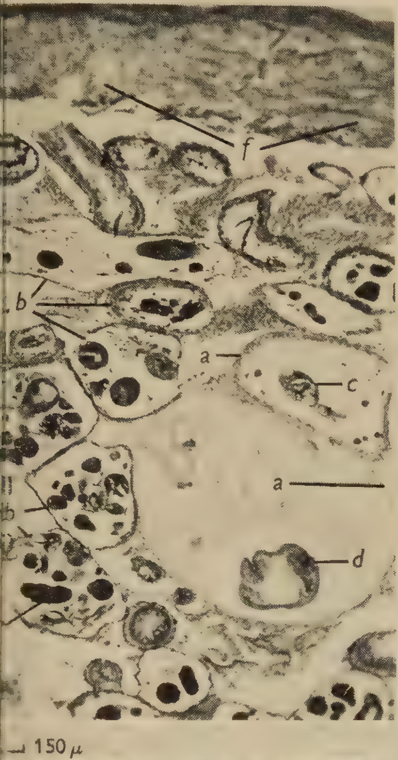
Fig. 1. Section through the upper part of the foot of *Cardium edule* L. showing dou trematode infection; *a*, sporocyst of *Cercaria fulbrighti*; *b*, sporocyst of '*Bucepha* *haimeanus*'; *c*, fork-tailed stage of *Cercaria fulbrighti*; *d*, *fully developed* stage of *C. f* *brighti*; *e*, cercaria of '*Bucephalus haimeanus*'; *f*, muscle layer of foot.

Fig. 2. Tail-less cercaria before spines develop.

Fig. 3. Characteristic markings on body of cercaria before spines develop.

Fig. 4. Part of sporocyst showing both tailed (*a*) and *fully developed* (*b*) cercariae.

Figs. 1 and 3 have been made from sections stained with Ehrlich's haematoxylin and eosi Figs. 2 and 3 were made from living material compressed between glass slide and cover-gla with Fig. 3 being made under oil immersion ($\frac{1}{12}$).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b
a
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a
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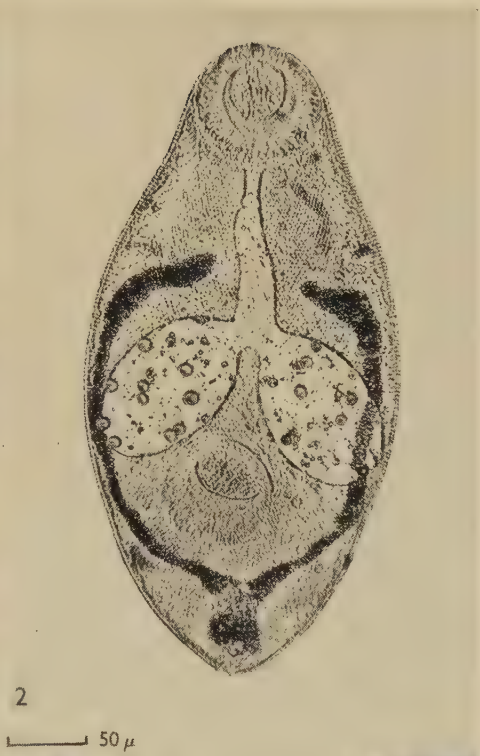
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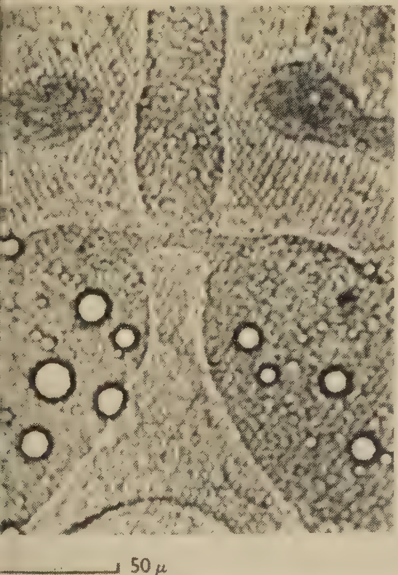
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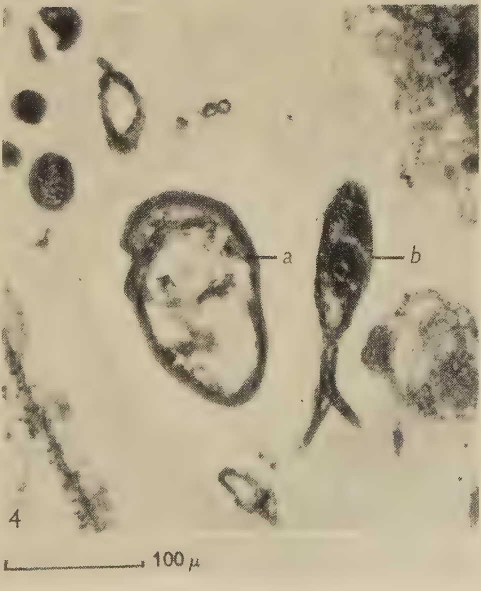
a
b
4
100 μ

NOTE ON SEA TEMPERATURES IN THE ENGLISH CHANNEL, 1921 TO 1949, AND PLYMOUTH SUNSHINE AND LIGHT

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From the Plymouth Laboratory

(Text-figs. 1–3)

rnational hydrographic station England no. 1 (E1) has been visited llarly for many years, and since 1921 the periods have been (nominally) a month, or more frequently. Much work has been done on the plankton chemical changes in the water, but no biological use has been made of temperature-depth observations, which are primarily of hydrographic ortance. The aim of this paper is to render the sea-temperature observa- over this long period readily available and to place beside them rvations on air temperature, sunshine and light. Such comparisons may xpected to give information upon the causes of variations in temperature, he adequate investigation of this very difficult problem is outside the e of this note. The subject is worthy of fuller treatment on the lines ested by Harvey (1925), based on data for the years 1921 to 1924, and has considered on broad lines by Sverdrup, Johnson and Fleming (1942).

The Sea-temperature Observations

hese are made with standardized thermometers in a Nansen-Petterssen r-bottle at 5 m. intervals down to an almost constant reading, and there- at 10 or 20 m. intervals to 70 m. with the usual precautions. Surface ples are also taken with a wooden bucket, the bottle observations at the ice being termed 0·5 m., but these two are here grouped together. Mean mn temperatures are based on means for 2·5, 7·5...67·5 m. The observa- are thus for single days, or possibly means for 2 or 3 days, in each month.

The Air-temperature Observations

hese are got from the Meteorological Office publications. In 1921 until end of 1922 the figures for daily temperature taken were those of the ough Meteorologist, which were based on observations at 9, 15 and 21 hr. station is on the Hoe, close to the Marine Biological Laboratory, and is bout 30 m. above sea-level. From January 1923 the Hoe records were d on mean values of the daily maximum and minimum readings taken at

21 hr. Accordingly, we took those read at 7, 13 and 18 hr. at the Cattewat station, Mount Batten, which is about 22 m. above sea-level. These cor tinued until after the end of our 1938 period. For 1947 until August 194 they were taken at Mount Wise, also about 22 m. above sea-level, and fro September 1948 till now they have been again at Mount Batten. Our pos war period means are based on observations at 3, 9, 15 and 21 hr. The seri is not therefore entirely concordant, though the resultant change is probab unimportant.

It is obvious that temperatures obtained, as monthly means, on land a not truly comparable with observations made over 20 miles to the south, sea. But they are the only ones available and with the slower changes m with at sea as compared with on land, the less frequent readings at sea st give a tolerably accurate representation.

The Sea and Air Temperatures

The minimum sea temperature for the 21 years, 7·4° C., was on February 1947, and the warmest winter minimum was in 1949, 9·9° C., 13 April. Excluding the exceptional value for 1947, ten winter minima l between 8·2 and 9·0° C., mean 8·55° C., and ten between 9·0 and 9·9° C mean 9·41° C. The mean for the twenty is 8·98° C. There was no indicati of any regular movement in the value of the minimum temperature whi occurred once in January, twice in February, sixteen times in Marc once in April and once in December.

In winter, surface and column temperatures are almost identical. T column had become isothermal by October in 13 years, and was nearing th condition in eight Septembers. In 1922, it was reached on or before 11 Ju and again by 22 September. The vertical mixing indicated is important bringing up the nutrient salts.

The air temperature monthly mean minima were never in January, b nine occurred in February, four in March, one in November and seven December, arranged quite irregularly.

The lowest maximum of the sea column was 13·12° C., and 5 years we below 14·0° C. Thirteen lay between 14 and 15° C., three 15° C. or ov with maximum 15·8° C. The mean column maximum is 14·34° C.

The maximum column temperatures occur when the column becom isothermal or nearly so, usually late in September or early in October. T maximum surface temperature was observed fifteen times in August, fo times in July and twice in September, such figures being influenced by t dates on which it was possible to make cruises. Mean monthly air maxi were twice in June, nine times each in July and August and once in Septemb

Table I shows the observations from which Fig. 1 has been construct Fig. 2 shows the mean temperatures of the water column at E 1 for select years which include the warmest and the coldest.

3 shows the column maximum and minimum temperatures for each he greatest range was for 1947, 7·02° C., and the least, 4·32° C., for Out of the 21 years the order of difference was: 1923, 21; 1925, 20; 19; 1924, 18; 1926, 17; 1927, 16, and in all these years the phos- content of the water was relatively high.

es for sunshine and light are also shown in Fig. 3.

TABLE I. COMPARISON OF SEA AND AIR TEMPERATURES

	Air temp., °C., monthly mean			Station E 1, column temp., °C.			Station E 1 Surface max.
	Coldest	Warmest	Mean	Coldest	Warmest	Mean	
n.	4·2	9·5	7·3	9·0	11·3	10·2	11·2
b.	0·9	8·5	6·4	7·4	10·2	9·3	10·2
ar.	5·9	9·5	7·4	8·3	10·0	9·0	10·1
r.	7·9	10·7	8·9	8·2	10·0	9·4	10·6*
y	10·5	14·5	12·0	9·25	11·4	10·4	13·5
e	13·8	17·4	14·9	10·5	12·4	11·5	15·7
y	14·7	19·9	16·3	12·1	13·8	12·8	18·4
g.	14·8	19·2	16·5	12·7	14·5†	13·6	19·4‡
pt.	13·1	17·0	15·1	12·8	15·2	13·8	18·3
t.	10·6	15·1	12·2	13·1	15·8	13·8	16·4
v.	5·8	11·1	9·0	11·9	15·0	13·0	14·6
c.	3·5	10·5	7·4	10·0	13·1	11·5	12·9

rface 11·2, 30 April 1930.

umn temperature for 29 August 1947 was 15·3° C. and the September and October were for 1947 also. For 1921, October, November and December gave respectively ·0 and 13·1° C.

August 1949.

Hours of Sunshine

values are those for Plymouth Hoe, as published by the Meteorological in hours daily. The yearly means vary from 5·54 in 1949, followed by 1929, 5·17 in 1933, 5·13 in 1921, 5·00 in 1948 to 4·03 hr. in 1931, other lues being 4·16 for 1927, 4·19 for 1924, 4·21 for 1947, and 4·25 for both nd 1938. The period covered was 1921–40 inclusive and 1947–49. The value for 23 years is 4·63 hr. a day. It is obvious that the heat received he sun is not linearly proportional to the hours of sunshine, since the ity is influenced by solar altitude, which varies throughout both year ay.

Illumination

e light falling on and penetrating the sea is absorbed and converted eat, the illumination affects the temperature. What is measured is the ht, from sun and sky, received on a horizontal surface on the laboratory an almost unobstructed position. The photoelectric cell used is more ive to the short than to the long wave part of the spectrum, so that the of cloud in obscuring the sun is less than with a thermopile, which is ximately uniformly sensitive throughout the spectrum. The current is red with a Cambridge thread-recorder galvanometer and the illumina- s got from the area of the daily chart, in kilolux-hours, which for the

year may be conveniently given in megalux-hours. The details of the measu
ments and results are given by Poole & Atkins (1935, 1936), Atkins (193

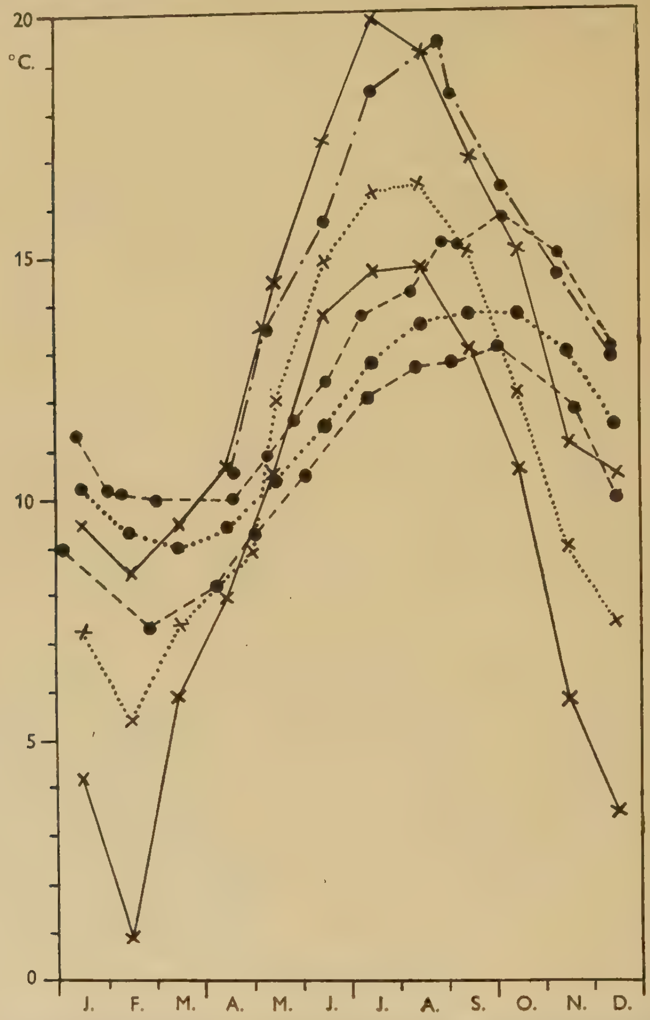


Fig. 1. Air temperature at Plymouth, monthly means (crosses). The continuous lines s
the warmest and coldest months of the period studied, plotted as if for single hot
cold years. The dots show the mean values. Sea temperatures at station E 1 (dark circ
are shown by broken lines for the water columns, warmest and coldest, as for air; the
and dashes denote the warmest sea surface temperatures encountered each month.
dots show the mean values for the water column over the 21 years.

Atkins & Ellison (1947), Atkins & Jenkins (1952). The observations co
14 years, 1930–40 inclusive, also 1947–49. The value for 1930 proved to
exceptionally high, 150 megalux-hours, but all efforts to show that this

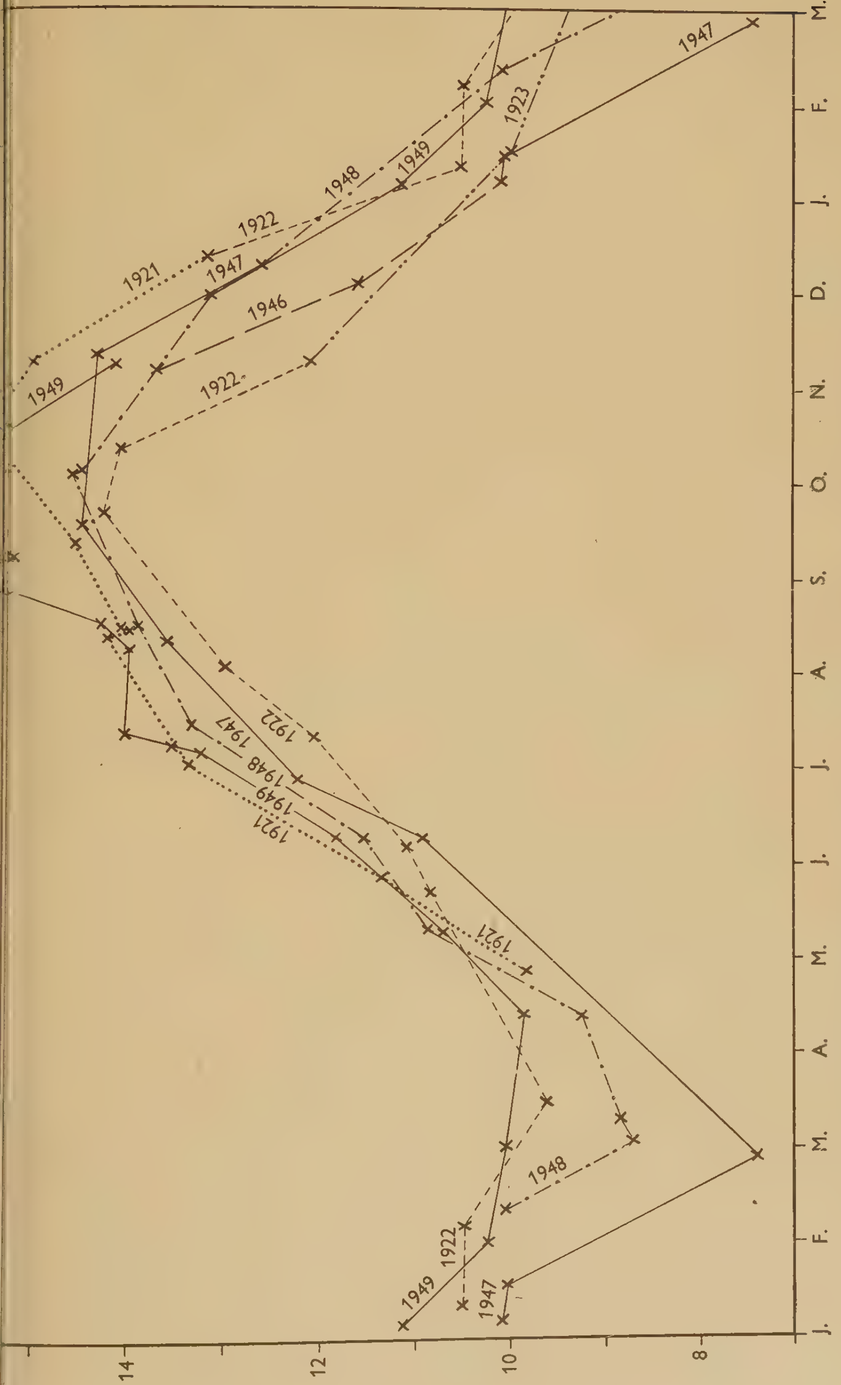
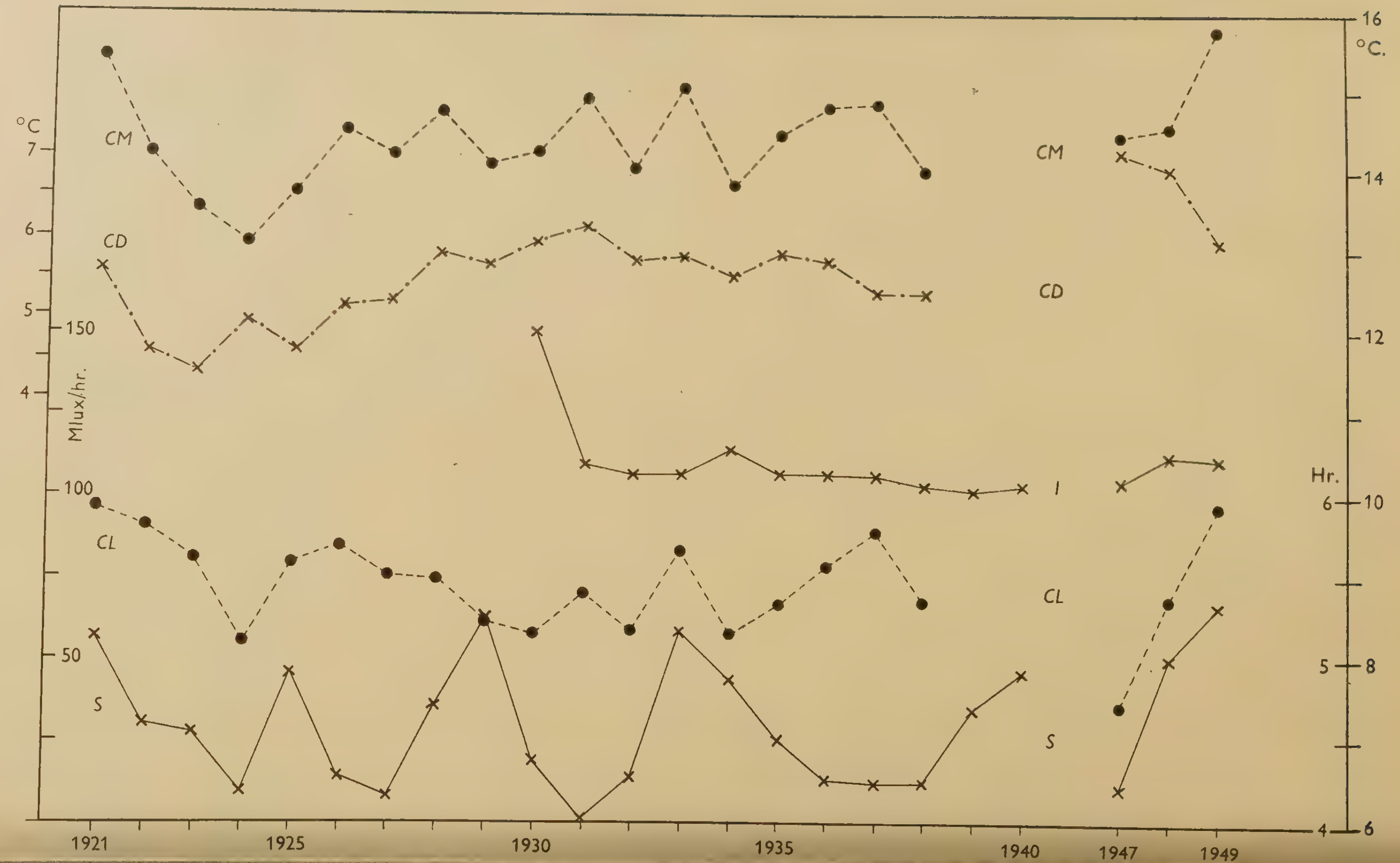


Fig. 2. Mean temperatures of the water column at E 1 for warm, cold and intermediate years.


°C
7
6
5
4
150
Mlux/hr.
100
50
CM
CD
CL
S
I
1921
1925
1930
1935
1940
1947
1949
16
°C.
14
12
Hr.
6
10
5
8
4
6

o a subsequent loss of cell sensitivity have failed, and between highest owest values for daylight Aurén (1933, 1939) got a similar high range Stockholm in a series between 1928 and 1937. The minimum was in , with 103 megalux-hours and the 14-year mean is 116 megalux-hours.

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URTHER EXPERIMENTS ON BIOLOGICAL DIFFERENCES BETWEEN NATURAL SEA WATERS

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From the Plymouth Laboratory

(Text-fig. 1)

previous paper it was shown that the eggs and larvae of a sea-urchin and vo species of polychaetes 'developed abnormally, or were in poor health, a water collected from the English Channel in the region of the Eddystone, ugh in water collected from the Celtic Sea development was generally al and healthy' (Wilson, 1951, p. 18). It was suggested that the Channel r lacked some constituent necessary for normal healthy development h was present in water from the Celtic Sea. In a mixture of the two waters arvae did almost as well as in the Celtic Sea water alone.

had been planned to continue experimental work on the properties of two waters when it became evident that further cruises to the westward tain Celtic Sea water would not be possible for some time to come. As lternative to using Celtic Sea water it was thought that water from the of Clyde, which like that from the Celtic Sea is generally characterized ne presence of *Sagitta elegans* (Russell, 1939; Barnes, 1950), might show iar properties, and be equally suitable for the purpose. Through the kind peration of the staff of the Marine Station at Millport, and particularly of H. Barnes, it has been possible to undertake some preliminary experi- ts using water from the Firth of Clyde. In these experiments the onsibility for the chemical work was undertaken by F.A.J.A. who has ared an account of the technical details (see pp. 336–8). The form of the riments, especially the second and third, was agreed upon after discus- in which Dr L. H. N. Cooper and Dr C. P. Spencer sometimes took

GENERAL METHODS

hods followed closely those already described for the earlier experiments *Echinus esculentus* L. (Wilson, 1951, p. 5). The concentration of larvae less than in most of the previous experiments, but more were used than he thin cultures of Exp. 8B (Wilson, 1951, p. 12). Decanting, to separate mming blastulae from undeveloped eggs, was unnecessary, as in 1950.

Pyrex crystallizing dishes of 7 cm. diameter were again used, the quantity water tested in each being equal at about 100 ml.

The Clyde water was collected in carboys cleaned at Plymouth and was sen by passenger train.

CHEMICAL WORK

Cleaning of Apparatus

We think that the attention given to this matter has been justified by the uniformity larval growth in each set of dishes, and that space may properly be given to a detaile description of the methods used.

The 5-gallon glass carboys in which the sea water was collected and kept unt filtered were cleaned beforehand by thorough scrubbing inside with a bottle brus followed by rinsing with 1 : 1 sulphuric acid as hot as seemed safe (about 60° C. The acid was removed by repeated washing with tap water. Finally, the carboys we washed three times with distilled water, and allowed to drain upside down. Th criterion of cleanliness for these carboys and all glassware used in these experimen was that on draining there should be no 'water-break', i.e. that the film of water o the glass spread evenly and did not gather into drops as on a greasy surface. If an article showed a 'water-break' the cleaning was repeated.

Crystallizing dishes, except those cleaned with chromic acid, were immerse completely for about 5 min. in 1 : 1 sulphuric acid at about 120° C., and then rinse successively with hot tap water and distilled water. They were allowed to drain an dry while inverted on an arrangement of glass plates and rods. During cleaning the were held in a fork of glass rod, and were not touched by hand.

The dishes cleaned with chromic acid cleaning mixture were allowed to stand i the cold mixture for 3 days, after which they were washed in running tap water unt the yellow colour disappeared. They were then allowed to stand in clean tap water fo 2 days, rinsed with tap water and distilled water and dried in the same way as the oth dishes.

Other glassware used was cleaned in hot 1 : 1 sulphuric acid and rinsed with ta and distilled water, contact with the hands being always avoided. The larger beake and vessels used for collecting and treating sea water were cleaned on the inside onl

The Berkefeld filter candles and the rubber and glass tubing used with them ha been used for earlier experiments. They were washed as thoroughly as possible wit hot tap water and distilled water, sterilized at low pressure in an autoclave, and re washed with boiling distilled water. The first litre or so of sea water filtered wa always discarded.

Measurement of Hydrogen-ion Concentration

In the first experiment, pH was measured with a glass electrode and the Cambridge Instrument Company's portable pH meter. For the later experiments th method was inconvenient, and pH was estimated by visual colour comparison wit Palitzsch buffers, using cresol red and thymol blue as indicators, applying the appropriate salt error corrections given by Buch & Nynäs (1939). When examining th dishes containing larvae it was found to be quite easy to remove 2 ml. with a pipett without much disturbing the water.

Alteration of Hydrogen-ion Concentration of Sea Water

This was done empirically by adding acid or alkali to portions of the filtered Clyd water, and aerating to bring the water into equilibrium with the carbon dioxide of th atmosphere.

ne reagents used were 0·1 N solutions of hydrochloric acid and sodium carbonate e from analytical grade chemicals; the quantities used were so small as to make it kely that the small changes brought about in salinity or trace elements are signi- t. Aeration was by means of air filtered through cotton-wool and passed rapidly ugh the water with a sintered glass gas distribution tube.

n adding acid to lower the pH, equilibrium with the CO_2 in the air, i.e. removal of came about after some 10 min. aeration. Trial experiments in raising pH with um hydroxide showed that the reverse process (absorption of CO_2) was much er, equilibrium not being obtained after an hour's aeration, and that it was better e sodium carbonate as the alkali. Even so, equilibration was slow, and had to be med to be complete when 30 min. aeration failed to cause any change in pH.

he filtered, but not specially aerated, water was at approximately pH 8·0. Solutions itable hydrogen ion concentrations were made by using the quantities of acid and i listed in Table I.

TABLE I

Acid or alkali added	pH after aeration
6 ml. 0·1 N HCl/l.	7·82
1 ml. 0·1 N HCl/l.	8·02
2 ml. 0·1 N Na_2CO_3/l.	8·14
4 ml. 0·1 N Na_2CO_3/l.	8·18
6 ml. 0·1 N Na_2CO_3/l.	8·28
8 ml. 0·1 N Na_2CO_3/l.	8·45

Treatment of Sea Water with Carbon

he procedure was suggested by the successful method used by Braus, Middleton alton (1951) to extract organic substances from drinking and river waters with e carbon. The particular make ('Nuchar C 190' unground) of carbon used by these ers does not seem to be obtainable in this country. On the advice of Messrs liffe, Speakman and Co. Ltd., we tried their own carbon 207B, 12–22 mesh, nerous sample of which this firm supplied without charge.

l. of the sea water to be treated was driven by air pressure at the rate of 1 l./hr. through a Berkefeld filter candle and then upward through a glass tube (length m., int. diam. 11 mm.) containing 10 g. of the carbon. This almost filled the tube. particles of the carbon were retained by a plug of cotton-wool at the top. The ed water was collected and kept.

Preparation of Extracts

he carbon through which the sea water had passed was washed by passing 50 ml. istilled water through the tube to remove some of the salt and was then spread large clock glass and dried at 60° C. for 4–5 hr. It was then put into a porous dum thimble previously ignited and extracted with acetone, and was extracted 5 hr. in a Soxhlet apparatus with 100 ml. of reagent grade acetone. This solvent used in the hope that it would extract more water-soluble material than would r, which was used by Braus *et al.* (1951).

pale greenish yellow acetone solution was obtained. Some partially charred erial was seen at the liquid level of the extraction flask where it had obviously been omposed by the heat of the heating mantle used for the apparatus. After filtration ugh an acetone-washed No. 43 Whatman paper to remove particles of carbon, the act was evaporated to dryness at low temperature in a 100 ml. beaker and dried n oven at 60° C. and weighed. A blank extraction was also performed on a 10 g. ion of the carbon. The weights and appearance of the extracts are given in Table II.

20 ml. of distilled water was added to each beaker, and the mixture warmed to 30–40° C. to help to break up the residues. That from the E 1 water dispersed almost at once to give a cloudy pale yellow-green solution, a small amount of greenish brown matter remaining on the bottom. That from the Clyde water did not disperse so readily, and left a larger amount of the greenish brown matter on the bottom of the beaker; the supernatant liquid was a clear yellow green, but paler than from the E 1 water. The film of residue from the blank extraction did not appear to dissolve in water.

By rubbing the undissolved residues with a glass rod they were dispersed in the liquid as quite small particles, and the extracts were allowed to stand for 3 hr., stirred, and each divided into equal portions of 10 ml. On adding these to the E 1 and Clyde waters, markedly turbid solutions were given by the E 1 extract, slightly turbid ones by the Clyde extract and clear ones by the blank extract. They were used without further filtration.

TABLE II. RESULTS OF ACETONE EXTRACTS

	E 1 water	Clyde water	Carbon blank
Vol. water used (l.)	20	20·5	—
Wt. of extract (g.)	0·0282	0·0222	0·0007
Appearance	Waxy greenish yellow solid with feathery brownish cystals together with some whitish opaque matter	Waxy greenish brown solid with feathery brown crystals together with some whitish opaque matter	Thin film of whitish deposit

Addition of Ascorbic Acid to Sea Water

The contents of a 1 c.c. ampoule of 'Celin' Vitamin C, Glaxo, stated to contain 100 mg. of ascorbic acid B.P. were diluted to 100 ml. with distilled water. 10 ml. of this solution were added to 1 l. each of filtered Clyde and E 1 waters. At the time of preparation these solutions should therefore have had an addition of 10 mg./l. of ascorbic acid.

EXPERIMENTAL RESULTS

Experiment I

Designed to test three waters, namely water from the international hydrographical station E 1, about 10 miles W.S.W. of the Eddystone, water from close inshore, and Clyde water. The first would come into the category 'Outside water' as used in the previous paper. In this paper it will be referred to by its international station number.

E 1 water collected from the sea surface by means of a wooden bucket. Strained through 200-mesh bolting silk.

Position: 50° 02′ N., 4° 22′ W.

Date: 17. iii. 52.

Ship: M.F.V. *Sula* with Dr L. H. N. Cooper.

Salinity: 35·25 ‰ pH (on 21. iii. 52) 8·28.

Inshore water collected by the same method and on the same day as E 1 water.

Position: ½ mile W.S.W. Rame Head.

pH (on 21. iii. 52) 8·25.

Clyde water collected from sea surface by means of a glass breffit on string handle Strained through 200-mesh bolting silk.

osition: approx. mid-channel between Keppel and Fairlie.
ate: 17. iii. 52. Time 15.00 G.M.T. (high-tide 15·26 G.M.T.).
mall boat, with Dr H. Barnes.
alinity: 32·59‰ pH (on 21. iii. 52) 8·17.

he sea-urchins were trawled on 20 March 1952 and kept under circulation rnight. A fertilization from a selected specimen of each sex was made in ixture of E 1 and Clyde water at noon the next day. The fertilization was ded equally into three beakers, and each portion washed with six changes ach of the three waters. Five dishes of each water, and five each of equal umes of Clyde water with E 1 and with Inshore waters were tested.

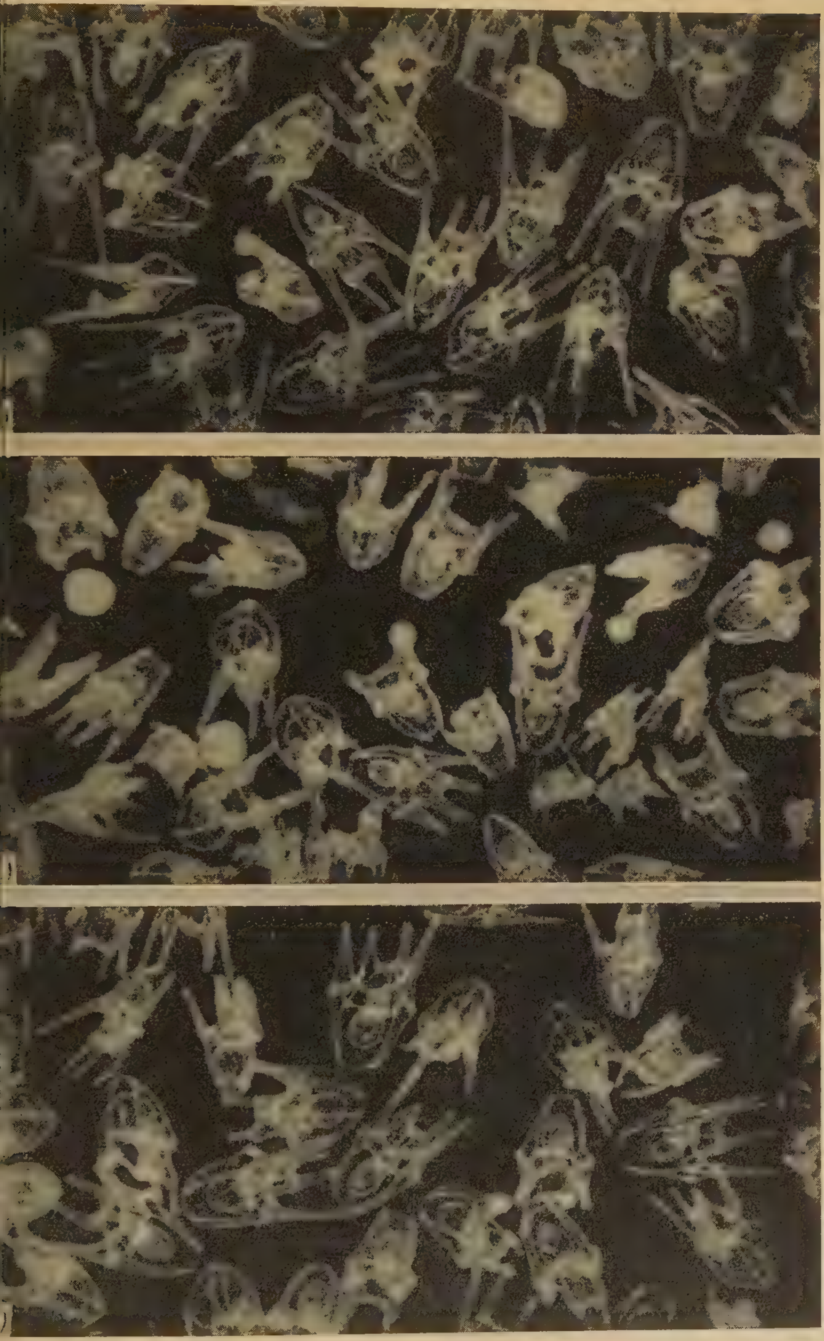
he results are summarized in Table III. The larvae in the Clyde water much better than in the E 1 or Inshore waters, and although the difference not as spectacular as in some of the earlier experiments it was none the well marked by the end of the experiment. The difference was, indeed, ceptible as early as the first day and by the third day was unmistakeable. that day (24. iii. 52) the larvae in the Clyde water had longer arms and e larger and finer looking than those in the E 1 and Inshore waters. It uld be emphasized that up to this time in any one set all five dishes were ntical one with another. Afterwards there were slight variations between nes in a set but they were only of small magnitude. In any dish of Clyde er the larvae were always much better than in any dish containing E 1 or hore water, though one E 1 dish in particular had from 25 March onwards her better larvae than the other four.

n 24, 25 and 26 March larvae were removed from some or all of the dishes closer examination after fixation, and for photographic recording. Careful nparison of the photographs fully confirmed the result already arrived at examination alive. Three of the photographs are reproduced here (Fig. 1). ey were obtained by the following method. With a glass dipper each dish a set of five was thoroughly stirred and one dipper-full withdrawn. The nples from all five dishes were mixed and the larvae then killed by the lition of a small volume of 5 % neutral formalin in sea water. The mixed nple was poured into a boiling tube and allowed to settle. When all the vae were on the bottom they were withdrawn in a pipette and transferred a glass dish. A crowded group was chosen at random and photographed. comparing these photographs it should be borne in mind that the group m the E 1 water looks better than it should do. Many larvae had already d and decayed leaving little trace, and the photograph is therefore biased favour of the living and the partially decayed dead. Most of the better-king larvae in this E 1 group came from one particular dish, mentioned ove. The other photographs are a fairer assessment of the conditions, for y few larvae had died in the Clyde, or the mixed Clyde and E 1 waters.

After 26 March, the last day recorded in the table, there was greater iation between dishes, but for another 3 days, until the experiment was

TABLE III. EXP. I. FERTILIZATION OF *ECHINUS ESCULENTUS* MADE ON 21 MARCH 1952

	Clyde water	E 1 water	Inshore water	Mixture of Clyde and E 1 waters	Mixture of Clyde and Inshore waters
22. iii. 52	Most blastulae swimming strongly at or near surface	Blastulae dispersed downwards a little more than in Clyde water with more on the bottom	Blastulae swimming a little more strongly than in E 1 water	Similar to blastulae in Clyde water	Similar to blastulae in Clyde water
23. iii. 52	Larvae a little more finely developed than in E 1 water	Larvae again dispersed downwards a little more than in Clyde water	Larvae still swimming a little more strongly than in E 1 water	Larvae a little more finely developed than in E 1 water	Larvae a little more finely developed than in E 1 water
24. iii. 52	Larvae larger and better formed than in E 1 water	Fewer at the surface and more on the bottom than in Clyde water	Similar to larvae in E 1 water	—	—
25. iii. 52	Well-formed plutei swimming well. Very few dead or abnormal	Plutei shorter-armed than in Clyde water, mainly swimming well. A moderately large number dead or abnormal	Plutei closely resemble those in E 1 water, but there are fewer dead or abnormal	Well formed plutei swimming well, like those in Clyde water. Very few dead or abnormal	Good plutei swimming well, like those in Clyde water. Very few dead, a few abnormal
26. iii. 52	A fair number of long-armed plutei swimming in mid-water and at surface but majority on bottom, some of these becoming short-armed. A very few dead	A small number of medium-armed plutei swimming, but majority dead on bottom, mostly well decayed. Those still living mainly short-armed	Similar to E 1 water but not so many dead. Plutei mostly short-armed and slightly inferior to those still living in E 1 water	A fair number swimming but majority on bottom. Larvae in structure similar to those in Clyde water. A very few dead	A fair number swimming but majority on bottom. In structure similar to those in Clyde water. A very few dead



1. Photomicrographs of larvae from Exp. I. Taken on 26 March 1952, a few minutes after fixation with dilute neutral formalin. ×35. (*a*) larvae from five dishes of Clyde water; (*b*) larvae from five dishes of E 1 water; (*c*) larvae from five dishes of Clyde and E 1 waters mixed in equal proportions.

ended, the larvae in the Clyde dishes and in the mixtures were better tha those still living in the E 1 water, where almost all were dead.

The larvae which survived in Inshore waters were, on the whole, better i the end than those in E 1 water (except for one dish of the latter) but were no as good as in the other waters. Throughout this experiment the room temperature varied between 15·1 and 16·6° C.

Subsidiary Experiment concerning Method of Cleaning Glassware

Before the experiments began it was suggested to us that the cleanin of the glassware with hot sulphuric acid was unnecessarily dangerou Arguments were advanced in favour of using potassium bichromate an strong sulphuric acid in the cold—a method commonly adopted for cultur glassware. We ourselves agreed that this method of cleaning would be safe and easier to carry out, but we thought that there was a risk that som chromium compound might be left adhering to the glass, even after thoroug washing, and that the results might thereby be affected. There was therefor added to Exp. I a set of dishes cleaned in this way to see whether there wa in fact, any difference between the two methods of cleaning.

Six bichromate-cleaned dishes were used, three for Clyde water and three for E 1. They were filled in the same manner as those cleaned with pure aci and stood alongside them. Within 24 hr. it was seen that the larvae in Clyd water in bichromate-cleaned dishes were swimming less strongly than i Clyde water in pure acid cleaned dishes. On 25 March one of the bichromat dishes contained well-formed plutei similar to those in the five pure aci cleaned dishes, but in the other two the plutei were very malformed an a considerable number were dead. The larvae in E 1 water in bichromate cleaned dishes differed little in appearance from the abnormal short-arme plutei in the other E 1 dishes, but by 25 March a higher proportion had die From these results it may be concluded that it is definitely unwise to us a bichromate cleaning mixture for glassware in which delicate larvae are t be kept.

The Influence of Hydrogen-ion Concentration

In the earlier work little attention had been paid to the pH of the sea wat used. On one occasion, when there was a marked difference between larva in waters of different kinds, the pH had been checked and found to be th of normal sea water in all dishes (Wilson, 1951, Exp. 5, p. 7). Among vario suggestions put forward after publication of this earlier work was that th results obtained might have been due to initial pH difference of the wate concerned. Smith & Clowes (1924—we are indebted to Mr F. R. H. Jon for this reference) have shown that with *Arbacia* and *Asterias* eggs th velocity of cell division is reduced in sea water adjusted to abnormally hig or low pH and that some retardation occurs even at pH 7·6 and 8·5. 'It doubtful if normal development can be obtained throughout a period of 24 h lutions more acid than pH 7·8 or more alkaline than 8·4' (Smith & es, 1924, p. 331). Although these figures are almost outside the limits to be obtained for normal sea water it was obviously desirable that the nce of pH be considered.

r Exp. I, described above, the initial pH of the waters after filtration he pH of the waters in the dishes after some days was determined with llowing results:

	pH	
	21. iii. 52	25. iii. 52
Clyde water	8·17	8·08
E 1 water	8·28	8·06
Inshore water	8·25	8·06
Clyde + E 1 water	8·20	8·07
Clyde + Inshore water	—	8·07

will be observed that there were initial differences between the various s, but that later on these differences were not so great. Although they relatively small, they none the less underline the desirability for a more e investigation.

Experiment II

is was devoted solely to testing the influence of pH within the range for natural sea water.

de water (collected 17. iii. 52) was adjusted (see pp. 336–7) to six different nities. A fertilization from one male and one female sea-urchin, newly d, was made at 6 p.m. on 31 March in water of pH 8·09, and washed our changes of the same water. The eggs were distributed in the usual

TABLE IV. pH OF EXPERIMENTAL DISHES

et	31. iii. 52	1. iv. 52	2. iv. 52	3. iv. 52	4. iv. 52
I	7·82	7·80	7·80	7·74	7·76
II	7·94	7·92	7·86	7·80	7·84
III	8·09	7·99	7·94	7·86	7·87
IV	8·16	8·02	7·98	7·91	7·94
V	8·28	8·05	8·04	7·98	7·99
VI	8·38	8·17	8·10	8·05	8·04

er, there being five dishes of water of each alkalinity. After distribution of water were pipetted from each dish and the pH of the combined ishes determined. This was also done on the following days. Table IV the results.

will be observed that in all instances the pH dropped more or less ly and that the drop was most pronounced in the higher alkalinities. e last day some of them showed a slight rise.

ween 4 and 5 p.m. on 1 April blastulae swam up in all dishes and no ences could be detected. At 10 a.m. on 2 April, however, it was notice- hat in set VI more larvae were on the bottom than in any of the others, while in set I there were slightly more larvae at the surface than in the othe Sets II–V appeared identical, with most larvae at the surface. The next da 3 April, no distinctions could be observed, all thirty dishes were alike wi about half the larvae at the surface, the others distributed in mid-water a over the bottom. Similarly, on 4 April all dishes seemed equally healthy, wi well-formed normal plutei all very much alike. There were hardly a abnormal specimens to be seen. At noon on that day larvae were remove fixed and photographed from all five dishes of sets I and IV–VI, the meth used being that already described (p. 339) for Exp. I. Careful examination these negatives, as well as of the fixed material with a microscope, revealed great distinction between any of them. The larvae from sets IV–VI appear identical, but those from set I had slightly shorter arms and were a little l developed than the others. The difference was very small and could scarc be observed in living moving plutei.

On 5 April the larvae in all dishes were dying, the flesh of the ar shrinking and the naked rods protruding, and by 6 April most of them w dead.

Throughout this experiment the room temperature varied between 1 and 16·5° C.

It is obvious that the pH differences had had no fundamental effect on ea development either as regards speed of the development or the structure the early pluteus, or on the time of death. In future work slight differen of pH between sea waters can probably be ignored.

Attempted Extraction of a Growth Factor: Experiment III

In pursuance of the hypothesis that waters which are favourable to devel ment contain some substance or substances lacking in those which are 1 an attempt has been made to extract, with the aid of active carbon, mater from the Clyde water and to add them to E 1 water. Details of the meth used are given above (pp. 337–8); it is only necessary to repeat here that in or to ensure complete control a similar extract was prepared from the E 1 wa and a blank extract from the active carbon alone. Water which had pas through the carbon was also tested. In all, ten tests with a set of five dis for each test were made. These are listed below, together with the p recorded on 24 April from samples of the original waters stored in g containers:

Set		pH
I	E 1 water	7·96
II	E 1 water + E 1 extract	7·94
III	E 1 water + Clyde extract	7·94
IV	E 1 water + blank extract	7·99
V	Clyde water	7·94
VI	Clyde water + E 1 extract	7·92
VII	Clyde water + Clyde extract	7·92
VIII	Clyde water + blank extract	7·87
IX	E 1 water through carbon	7·99
X	Clyde water through carbon	7·99

e waters used were newly collected as follows:

water collected from sea surface with wooden bucket. Not strained.
tion: 50° 02′ N., 4° 22′ W.
: 16. iv. 52.
: R.V. *Sabella* with F. A. J. A.
ity: 35·26‰.

le water collected from sea surface by dipping with a glass breffit. Strained h 200-mesh bolting silk.
: 16. iv. 52. Time, 11.00 G.M.T. (high tide 16·07 G.M.T.).
tion: mid-channel between Keppel Pier and Fairlie.
ll boat with Dr H. Barnes.
ity: 32·75‰.

e sea-urchins were trawled on 22 April and a fertilization from one male ne female was made at 5 p.m. the same day. A mixture of equal parts de and E 1 waters was used, the fertilized eggs being divided equally ch half washed in six changes of one or the other water. The eggs which ransferred to the waters which had been passed through active carbon urther washed in the carbon-treated water before being put into the . The glass dipper method of transference (Wilson, 1951, p. 5) ensured ery nearly the same numbers of eggs were put into each of the fifty shes.

he first examination on the following day (23 April) it was at once that in all dishes to which Clyde or E 1 extracts had been added I, III, VI and VII) the eggs had cleaved irregularly and had died in leavage. The extracts had proved poisonous.

ll the other sets the embryos were alive. At 9.30 a.m. the blastulae till within the fertilization membrane and motionless. It was observed most 100 % of the eggs had fertilized but that about 10 % had developed hat abnormally and it appeared that the fertilization was not quite as s those for Exps. I and II. Shortly after noon the blastulae, free from embrane, were beginning to swim up, noticeably more so in some sets n others (see Table V). At this early stage the blastulae in the E 1 water, ally in that which had been passed through carbon, lagged behind all hers, although those in the E 1 water to which the blank extract had dded were reasonably active. The blastulae in the Clyde water with extract led all the rest. After this early stage there was from time to ome variation, first one set taking the lead and then another. It was oticeable how all five dishes in any one set presented a uniform appear- By the fourth or fifth day there was relatively little to choose between , IV, V and VIII, although IV and VIII (the waters to which blank ts had been added) seemed to be slightly inferior to the other two. The tion was too subtle to indicate in the table. On 24 April the pH of one n each set was determined and is recorded in the table. On 26 April rvae were mostly good four-armed plutei, but they were not quite so eveloped in any of the dishes as those in the Clyde water of Exp. I of

TABLE V. EXP. III. FERTILIZATION OF *ECHINUS ESCULENTUS* MADE ON 22 APRIL 1952

		Set I E 1 water	Set IV E 1 + blank extract	Set V Clyde water	Set VIII Clyde + blank extract	Set IX E 1 through carbon	Set X Clyde through carbon
23. iv. 52	12–10 p.m.	Blastulae rising, but none at surface	Blastulae rising well, a few at surface	Blastulae well up, a fair number at surface	Blastulae well up, a fair number at surface	Blastulae all on the bottom	Blastulae rising well, a few at surface
	5 p.m.	Almost all at surface, few in mid-water or on bottom	Many at surface and in mid-water, fair number on bottom	Almost all at surface, few in mid-water or on bottom	Many at surface and in mid-water, fair number on bottom	A few at surface and in mid-water, majority on bottom	Most in mid-water and up to surface, many on bottom
24. iv. 52	4 p.m.	Many at surface, some in mid-water, many on bottom	Fair number at surface and in mid-water, many on bottom	Many at surface, some in mid-water, many on bottom	Fair number at surface and in mid-water, many on bottom	A few abnormal larvae swimming, almost all dead	A few at surface or in mid-water, majority on bottom, some abnormal
		pH 7·84	pH 7·84	pH 7·90	pH 7·84	pH 7·94	pH 7·89
25. iv. 52		Majority at surface and in mid-water, mainly normal, a few dead				A very few abnormals living. All others dead	A few in mid-water, all others on bottom, abnormal
26. iv. 52		No change				No change	No change
27. iv. 52		Majority on bottom becoming abnormal				No change	No change
28. iv. 52		Almost all on bottom very abnormal, some dead				Some of the few still living regenerating arms	A few dead, otherwise little change
29. iv. 52		No change				A few apparently normal plutei in all dishes, mainly on the bottom	A fair number dead, but majority still alive on bottom, abnormal

me age and of which photographs were available for comparison. There nore variation in form and there was a greater proportion of plutei mal in varying degree. By the next day all were becoming abnormal, was shrinking down the arms and exposing the rods, which eventually off. Not many larvae had died after another 2 or 3 days.

main interest of this experiment derives from the condition of the in the waters which had been passed through the active carbon, which nown (see pp. 337–8) to have extracted substances from them. In the n-treated E 1 water larvae showed little vigour and soon most of them dead. In carbon-treated Clyde water, on the other hand, the larvae ed more vigour in keeping up off the bottom, although after a few days of them were lying on it. They failed to develop long arms but most ned alive, stunted in their growth. Towards the end of the experiment of the few larvae still surviving in set IX, a few in each dish (one dish nore than the others), unexpectedly grew arms which they had not had usly and to all appearance were normal plutei. Others had partially arms. This occurred, it should be noted, at a time when in all the other the larvae had lost the arms which they had previously possessed. newly formed plutei, however, were very lethargic, lying on the ms of the dishes and rarely swimming. The water in the dishes was ly milky, probably from bacterial growth.

temperature of the room throughout the experiment was very uniform, g between 15·0 and 15·7° C.

Subsidiary Experiment with Ascorbic Acid

lier, Ray & Magnitzky (1950) of U.S. Fish and Wildlife Service found relation between the pumping rate of *Ostrea virginica* and a factor ally present in minute amounts in sea water which they designated as ohydrate' or some other compound quantitatively associated with the hydrates responding to the test.

a letter to Dr L. H. N. Cooper, dated 12 March 1952, Dr John Lyman that U.S. Fish and Wildlife Service investigators now believe that this ance is ascorbic acid (vitamin C) and Dr Lyman suggested that the h factor lacking in the Channel water might possibly be ascorbic acid. der to test this suggestion there were included in Exp. III a set of five of E 1 water to which 10 mg./l. of ascorbic acid had been added (see for details) and another set of five containing Clyde water with ascorbic Except that the blastulae in the E 1 dish with ascorbic acid swam up off ottom a little earlier than in the untreated E 1 water the behaviour and opment of the larvae in these waters dosed with ascorbic acid was closely r, throughout the experiment, to the larvae in the untreated waters.

is experiment cannot be considered conclusive. It was tried out in ater which proved better than usual and with a batch of eggs which may been of inferior quality (see p. 345).

Discussion

It has now been shown that sea waters from two distinct localities, nam the Celtic Sea and the Firth of Clyde, are sometimes more suitable laboratory rearings of *Echinus esculentus* than is sea water from the Engli Channel in the neighbourhood of Plymouth. Both of the better waters co from sea areas known to be normally characterized by a *Sagitta eleg* plankton, whereas the poorer Channel water supports only a less abund *S. setosa* community. No doubt the relative merits of the two sorts of wa vary from time to time. This seems to be indicated by those earlier expe ments in which the larvae failed to develop properly in both types of wat and by Exp. III of the present series. In this latter experiment the lar in the E 1 water were practically indistinguishable from those in the wa from the Clyde, although in neither was the rearing one of the best. The plu showed a greater variation of structure than usual, about half being to grea or lesser degree subnormal. This may have been due to variability in t condition of the eggs, but on this aspect the experiment was not controll Nevertheless, the larvae in the E 1 water did better than previously, implyi that at times the water occupying this Channel station will be fairly favoura for developing *Echinus esculentus*. That the larvae of this species do devel somewhere in the region is obvious from the numbers of adults present the trawling grounds (but see Wilson, 1951, p. 18). Late larvae of the spec were seen in plankton catches taken off Plymouth in May and June 19 but in that year no laboratory tests of the sea water were made.

The results of mixing waters (Exp. I) are most instructive. They probably most easily explained by assuming that the poor condition of t larvae in the E 1 and Inshore waters was due to deficiency of somethi which was present in the Clyde water. In previous experiments Celtic wa had appeared to supply some necessary ingredients to similar mixtures. T conjecture that the better waters contain some essential material which deficient in the poorer waters seems to be supported by the results of Exp. where the larvae did so badly in both good and bad waters after they had be passed through activated carbon. Although there is no definite proof, would seem more probable that the carbon removed needed subtances fr the water than that it added something deleterious. Indeed it is known t the carbon extracted a considerable quantity of matter which was recove with acetone (see p. 337). The acetone extracts from the carbon alone, the other hand, had little or no effect on the larvae in the waters to which t were added.

The much delayed outgrowth of arms on some of the few surviving lar in the carbon-treated E 1 water (set IX of Exp. III) is of particular intere It raises the question whether the early death and decay of the majority the larvae in these dishes had restored to the water, perhaps by bacte action, the material needed. Alternatively, perhaps, the larvae had obtain it in particulate form by way of the mouth.

SUMMARY

experiment sea water from the Firth of Clyde yielded better cultures
hinus esculentus larvae than did sea waters from two positions in the
h Channel.

m the Clyde water materials of unknown composition were extracted,
active carbon and acetone. The addition of these materials to both
and Channel waters resulted in the death of the *Echinus* eggs during
cleavage. A similar extraction from Channel water had the same effect.
Clyde and Channel waters from which these materials had been extracted
poor abnormal cultures, especially the Channel water in which most
died early. A few, however, survived, and later on some of these grew
lmost normal plutei.

s shown that alkalinities between pH 7·82 and 8·38 have little or no
on the growth and form of early *Echinus* larvae.

addition of ascorbic acid to both Clyde and Channel waters produced
or no improvement, but the experiment was in the main inconclusive.

me evidence is advanced that English Channel water is not always an
ourable medium for the development of sea-urchin larvae.

me glass dishes which had been cleaned with a cleaning mixture con-
g of potassium bichromate and sulphuric acid gave rise to abnormal
inhealthy larvae, while similar dishes cleaned with hot sulphuric acid
ot.

ails of the chemical methods used in these investigations are given.

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THE BOAR FISH, *CAPROS APER* (L.), AS A POSSIBLE BIOLOGICAL INDICATOR OF WATER MOVEMENT

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(Text-fig. 1)

s aper (L.) is sporadic in its occurrence in the English Channel; for years near Plymouth it has been scarce or absent. Between 3 June 1948, Capt. C. A. Hoodless began to keep exact records of all fish trawled by *Sabella*, and 29 June 1949 no *Capros* were taken by that ship. From ne 1949 to September 1951 odd fish were trawled in the summer months, more than three in a haul, and in October 1951 two more fish were t separately. Then on 30 October two hauls by R.V. *Sabella* about s south-west of the Eddystone brought up 1000 and 300 fish, followed on 13 November, 100 on 10 December and 50 on 17 December. In me area on 1 November M.F.V. *Sula* in two hauls caught 200 and 50, her 50 near Station E 1 on 14 November and 9 on 17 March 1952. All catches were in hauls of 45–90 min. duration. Single fish were taken h ship in February 1952.

ypothesis to explain the introduction of a considerable fresh stock of *s* into the English Channel late in the year has been developed from an hypothesis (Cooper, 1952*a*) concerning 'submarine eagres' in canyons continental slope.

a number of occasions between July and October inclusive the post- stages of *Capros* have been recorded by Russell (1930–47) and Corbin -51) from the 2 m. ring-trawl catches taken weekly 2 miles east of the tone. In all years in which young *Capros* occurred, except 1937, the s of the siphonophore, *Muggiaea*, occurring at about the same time was *chi* and not *M. atlantica*. *M. kochi* was the species present (Corbin, e communication) on 24 October 1951 at International Station E 1, miles from the position where the large hauls were made a few days There are reasons, as yet unpublished, for suspecting that *M. kochi* English Channel may indicate deeper water brought up over the nental slope lying to the south-west. These events therefore suggested *apros* might also indicate water from the continental slope.

he Plymouth aquarium *Capros*, by means of gentle fin movements, may n poised almost stationary in the water or make slow excursions around

nk. Occasionally it may make short random darts through the water. it does not appear the sort of fish to make far-reaching migrations by ming, the illusion of migration may be due to travel from some distant e of the water mass in which it occurs. If so it might be of value as ogical indicator of water movement.

cords of its distribution have been summarized by Cornish (1868), mbe (1879) and Legendre (1934). The latter took eighteen examples from omachs of germon or white tunny (*Germo alalunga*) caught in the Bay scay north-west of Concarneau. *Capros* has often been found in the chs of tunny. Apart from records near Plymouth or off Cornwall, all s are near to the western European or Mediterranean continental slopes. uthorities agree on a distribution epitomized by Holt & Calderwood) who said that 'it is known to frequent rather deep water, although in eighbourhood of Plymouth it is tolerably abundant in depths of less 30 fathoms during summer months'. In 1879, Gatcombe was told that n a few years *Capros* had become a pest, so much so that trawlers had obliged to change their fishing grounds to avoid them. So great was eight of the fish that the fishermen had to cut large holes in their nets them escape.

llett (1896) stated that it occurs along coasts in great numbers only after as. He was writing mainly of the Mediterranean where continental es are narrow and either 'submarine eagres' or 'capsizing of water es' would introduce *Capros* directly into coastal waters.

Danois (1913) said that it is a deep-water fish and that only accidentally it rise to the shallow banks of the English Channel. Later (1943, p. 142) ggested that the group containing *Capros* has evolved around coral In his constructive broad survey of the fauna of western European s he (1948, p. 278) classes *C. aper* as an inhabitant of the 'bordure nentale' and Atlantic slope and associated there with the coralline facies. corals he divides into yellow and white (or flesh pink, *rose chair*). rding to Le Danois the yellow coral, unlike the white, does not thrive in and prefers hard steep rocks. It is most often found on parts of the tic Slope which are *tourmenté et chaotique*. It would be the species of most likely to occur on the steep sides of submarine canyons. This ation should mean that *Capros* is indigenous in submarine canyons sted with yellow coral. In clear well-illuminated water the yellow or colour of *Capros* should provide camouflage amongst such yellow coral, probably has little value in the almost dark waters below 200 m. depth. wever, Day (1880) described fish which when first received in the minster aquarium from Mevagissey were of a rich red hue changing in ourse of a few days to straw colour. Their colouring may be banded or Evidently, if it so wished, *Capros* could adapt itself to match either of oralline environments described by Le Danois, if sufficiently illuminated.

Our present interest will be centred on the population of *Capros* which ma occur associated with yellow coral in the upper and narrower parts of submarine canyons between about 200 and 500 m. in depth.

A hypothetical submarine eagre, arising from vertical oscillations in stratified water following on-slope strong winds, has been suggested (Cooper 1952*a*) as running up a submarine canyon like a bore or eagre in the River Severn or Trent. In the upper, more constricted, part of the canyon thi submarine eagre is considered likely to become quite violent, sufficiently s to dislodge *Capros* from its environment against the coral encrusted walls to which the fish would be unable to return. It is scarcely credible that th momentum of such an eagre could carry the water and the fish in it th 200 miles from the continental slope to the neighbourhood of the Eddyston nor is that necessary. It is necessary only to believe that the eagre project the water sufficiently far on to the continental shelf for it to get caught up i the general cyclonic circulation of the Celtic Sea. The eagre would injec a limited mass, a 'bubble' or 'eagre spurt' of canyon water into shelf wate which could have quite different properties but which, none the less, woul become effectively labelled by the considerable population of *Capros*.

To be of value as a biological indicator *Capros* would need to be exceedingl abundant in its coralline home. However, it is not postulated that there i always a dependable uniform stock available to colonize submarine eagre The continental slope would seem likely to be an exacting environment fo species largely restricted to it, so that the population of *Capros* against th slope may fluctuate widely.

A canyon wall swept clean of *Capros* by a submarine eagre would be slowl re-colonized either by adults moving in from neighbouring less dissecte areas of the slope, including the muddy areas inhabited by 'white coral or by metamorphosis in the right place of larvae spawned from such areas There may be a difference in age composition of *Capros* populations in submarine canyons and in the Celtic Sea and English Channel, on the one hand and over the muddy white-coralline areas on the other.

A submarine eagre would introduce on to the continental shelf a compac population of *Capros*, but the water introduced with the fish might be o two kinds. An eagre spurt is visualized as having a compound structur After a persistent strong on-slope wind a homogeneous water-mass is likel to be present, not only within submarine canyons to several hundred metre depth, but also for some miles inward over the continental shelf. As the win dies down the water in the canyon may oscillate. The upper part which woul sweep out the stock of *Capros* on to the shelf need not be distinguishable fro the neighbouring shelf water. The event could not be followed by measurements of chemical properties or temperature but only by current meters o biological indicators. However, towards the end of a large and deep oscillation, when the canyon walls have been swept clean of *Capros*, deeper wate arkedly different properties and richer in nutrients should follow. The t may be compared with a comet, the head of the eagre spurt being cterized only by *Capros* and any accompanying biological indicators, the ontaining few *Capros* but distinguishable chemically.

e subsequent history of an eagre spurt on the continental shelf would nd on the general circulation, tides, bottom topography and weather. n, as often, the general circulation is cyclonic, *Capros* from the southern of the Celtic Sea would be carried towards Ushant, the English Channel Land's End.

metimes, but not always, eagre-spurt water would be slightly heavier water over the shelf where it would tend to fill the troughs in the ed. There *Capros* would be expected to be trawled in large numbers more in the troughs than on the banks. Such a distribution would be due to pography and not to the nature of the sea-bed.

currents are zero in immediate contact with the bottom and increase rength upwards and the strongest currents are tidal. According to Veen's formula (1936) the current at 4 m. off the bottom will be about that at 10 cm. so that tidal currents will tend to spread any dense lation of truly demersal fish. In the southern Celtic Sea the major and r axes of the tidal ellipses are almost equal so that there a circular shoal h would, as it spreads, remain almost circular. But in the English and ol Channels, the tidal streaming is almost linear, flood and ebb currents flowing in almost the same directions for several hours. The major axis e tidal ellipse much exceeds the minor. In these waters a dense demersal will not spread radially as in the Celtic Sea but into an elongated band. ngly swimming fish, such as the gadoids, may well choose to reform mpact shoal if this better suits their way of life but it is unlikely that *os* could do so. If the present hypothesis is correct shoals of *Capros* in western English Channel, but not in the Celtic Sea, are likely to occur as gated bands, aligned along the major axis of the tidal ellipse.

his theorem is worth investigation for a practical reason. All strictly ersal shoaling fish are likely to respond to this effect of the tide in some e. If therefore a trawler detects on its echo-sounder a shoal of fish near bottom, it is likely in the English Channel to make better catches by ng its trawl in or against the direction of the main tidal stream than by ng across it.

eagre spurt containing *Capros* in numbers will be subject not only to ading due to differential bottom currents but to attrition due to bottom on of all kinds. Odd fish would become distributed over a much wider than that of the eagre spurt which may be identified only while the lation remains dense.

ubmarine eagres can arise only in stratified water and stratification over continental slope, as elsewhere, is most strongly developed in summer.

Consequently, only then would shoals of *Capros* be injected on to the co tinental shelf to arrive off Plymouth some weeks later. Again, in shallo homogeneous water wind causes vertical and lateral mixing right to t bottom. In winter strong winds should disperse shoals of *Capros*. Althoug beneath a thermocline, strong winds may produce more mixing than h been hitherto realized (Mortimer, 1952), nevertheless, the forces tending disrupt shoals will be much less in summer.

TABLE I. STRONG WINDS IN THE CELTIC SEA, SEPTEMBER 1951

		Scilly Observatory Wind observed			Position 48° 30′ N., 9°–10° W. Estimated wind	
Date	Hours	Direction	Knots	Force Beaufort	Direction	Force Beaufor
13 Sept.	06	200	27	6	300	6
	12	290	31	7	280	6
	18	280	21	5	270	6
14 Sept.	00	260	17	5	250	5
	06	260	19	5	240	4
	12	220	16	4	220	6
	18	220	18	5	230	7
15 Sept.	00	200	26	6	220	7
	06	220	22	6	310	5
	12	320	21	5	300	4
	18	290	12	4	300	3
16 Sept.	00–18	250–300	6–8	2–3	—	—
23 Sept.	00	180	22	6	260	6
	06	270	13	4	280	6
	12	240	13	4	270	6
	18	260	23	6	270	6
24 Sept.	00	260	23	6	270	6
	06	240	20	5	240	7
	12	220	23	6	240	7
	18	220	22	6	260	7
25 Sept.	00	210	29	7	260	6
	06	230	18	5	270	5
	12	240	13	4	260	5
	18	240	12	4	280	5
26 Sept.	00	250	13	4	290	5

There is a critical wind speed at about 7 m./sec. (14 knots, Beaufo Force 4) (Rossby & Montgomery, 1935; Munk, 1947). Above this speed t effect of wind becomes 'hydrodynamically rough'. Only such winds can g hold of the water sufficiently to produce the conditions which must prece submarine eagre formation. The effect of wind of Beaufort Force 4 will trivial.

A minimum Force 6–7 (22–33 knots; 11–17 m./sec.) was visualized necessary to produce the cushion of light water against the slope, the upwa swing of which would produce a submarine eagre when the wind dropp Winds of this strength were recorded on 13, 15, 23 and 24 September 195 (Table I).

e nearest British Meteorological Station is at Scilly (St Mary's) about niles from the area where information is required. Every 6 hr. in the Weather Reports the anemometer readings there are reported for ion and strength in knots. These exact records have been extracted. the further reports from merchant and weather ships (including the h vessels at 45° N., 16° W.) and the 6 hr. synoptic isobaric charts, ments of wind direction and speed in knots at 48° 30′ N., 9–10° W. been made. They are printed in Table I as Beaufort numbers which spond better to their probable accuracy.

submarine canyons facing into the wind, submarine eagres could have created on 14, 15 and 25 September when the strong winds dropped. ull of about 12 hr. on 14 September may not have been long enough to be ive while any dense shoal of fish ejected on to the shelf then or on eptember would probably have been dispersed by the strong wind on d 24 September. It is suggested that on the following day, 25 September, the wind dropped to 13 knots at Scilly and did not exceed 18 or perhaps ots near the slope, an eagre spurt was injected on to the continental shelf a submarine canyon and that this brought up the population of *Capros* was found near the Eddystone on 30 October and subsequently. Such s were possible in a number of the canyons depicted by Beaugé (1934). e continental slope concerned runs from south-east to north-west, and es of most of the reputed canyons point south-west or south. In these, -westerly or southerly winds should be most favourable for creating s. However, one of the largest canyons at 48° 22′ N., 9° 30′ W., named e French Fisheries Department 'la Machoire du Sud' has an almost ht axis pointing west by south. Its southern wall has been defined by S. *Dalrymple* also. In this canyon, and in this alone, westerly winds create considerable submarine eagres. On 25 September a large eagre nore likely to occur there than in any other canyon. The winds most likely ave been responsible for the eagre spurt which brought in the *Capros* are printed in heavy type.

ce this was achieved quiet weather was needed to allow the shoal of *os* to reach the neighbourhood of the Eddystone intact. For the ining 5 days of September force-4 winds were consistently recorded at y. Thence, until 30 October, of 120 records of wind at Mount Batten, outh, only 12 were in the range 11–16 knots (force 4) and but one ed 17 knots. Such light winds would have little power to disperse an spurt travelling up-Channel. The meteorological records were therefore sely what the theory demands. If this view is correct, the shoal of *Capros* carried about 200 miles in the eagre spurt in 35 days, or about 6 sea s per day.

ater containing *Capros* in an eagre spurt may be heterogeneous. Only e water can be sampled in the middle of the shoal could distinctive hydrographical results be expected. Trawling does not generally enable o to know exactly where such a shoal was placed. Even if the centre of t shoal could be found, a high salinity characteristic of slope water is to expected only under very favourable conditions. Let us consider an unmix eagre spurt, fresh from a canyon, containing 10,000 *Capros* per standa trawl haul and having a salinity of 35·6 ‰ and a total phosphorus content 0·9 μg.-atom/l. Let us consider its admixture with enveloping water co taining no *Capros*, a salinity of 35·20 ‰ and total phosphorus amounting 0·5 μg.-atom/l. The mixed waters would have the properties set out Table II.

In October and November 1951 there was no change in the chemic properties of the water at the nearby station E 1 which could be associat with the incursion of *Capros*. Thus, if the hypothesis can be proved, *Capr aper* would provide a far more sensitive indicator of the presence of co tinental slope water near Plymouth than would any chemical determinatio

TABLE II

Percentage of		Number of *Capros* per standard trawl haul	Salinity (‰)	Total phosphorus (μg.-atom/l.)
Eagre spurt water	Enveloping water			
100	0	10,000	35·60	0·9
10	90	1,000	35·26	0·54
1	99	100	35·20$_8$	0·50$_4$
0	100	0	35·20	0·50

A criticism may be made that a density of fish in an eagre spurt as high that assumed in Table II is improbable since, for lack of food, deep-wat fish are rarely in sufficiently dense concentration. This may be especial true of a species that remains in much the same place. What would it eat? T rain of food from above would support a certain number but surely not many? In the open ocean this criticism would be pertinent, but furth investigation may show that it is not true against a continental slope whe food may arrive by means other than 'raining down'. There is abunda evidence (*inter alia* Cooper, 1952*a*, fig. 3, Station 51; and 1952*b*) that winter vertical homogeneity of water against the slope, which must be due vertical mixing, extends at least 3 to 5 times deeper than it does in the op ocean to the westward. This vertical homogeneity is a fact and not depende on the hypotheses of cascading, capsizing and submarine eagres which ha been erected to explain it. The vertical movement or mixing of the wate whatever its cause, will carry downwards some of the organisms which cann swim. The population of these in the 200–400 m. strata against the slope likely to be comparable with that in the surface layers. Again, there a numbers of strongly swimming vertical migrants near the slope, such *Sagitta serratodentata*, which feed near the surface at night and make diurn vertical migrations to several hundred metres depth. In the ocean again

inental slope—and only there—reserves of food for fish comparable
hose over continental shelves are likely to be present at considerable
. Probably, therefore, dense populations of fish at depth in these sub-
canyons may find adequate food at any season, and abounding food
cascading or after southerly storms. On this view the postulated
of 10,000 *Capros* thrown up by a submarine eagre and caught in
ard trawl haul does not seem so unreasonable.

e trawls and dredges are very inefficient instruments to sample fish
ing against the rugged walls of a submarine canyon, the only hope of
ing evidence of abundance in such a place would seem to be with an
-water camera, protected by a cage and triggered to take a photograph
the cage strikes a canyon wall.

ould be unreasonable to assert that eagre spurts similar to that here
sed did not occur in the nineteen-thirties. If they occurred they
t few *Capros*. Consequently, the present hypothesis is acceptable only
further assume that the stock of *Capros* over the continental slope
went a decrease in numbers comparable with that undergone by many
d invertebrates near Plymouth at the same time. Catastrophes to adults
s and larvae due to excessively violent capsizing might provide an
ation of short-period changes but are unattractive to explain long-
fluctuations. It is more probable that the water of the Atlantic Ocean
g the continental slope has been subject to vicissitudes similar to those
English Channel. We should then have to seek an understanding of
ents in terms of changes in the fundamental circulation of the Atlantic
.

hin the English Channel *Capros* would find conditions resembling its
ated indigenous home only on rocky declivities from which tidal
ts and breaking seas would tend to detach it. If a stock of the fish were
into the Hurd Deep, there against the steep walls it might find food
elter and thrive for a while. The Hurd Deep would then provide
re of dissemination of *Capros* eggs. Again, a passing shoal might
t a lodgement amongst the rocks of the Eddystone where they may
detected for some while. Eggs spawned there would be sampled at
ndard young-fish-trawl station 2 miles to the eastward. In similar
ouch (1863) mentioned that a Penzance trawler in July 1844 in a few
ught about 200 close into the Runnelstone. He says: 'These fish may
be found within half a mile of this well-known rock, where they are
ly induced to assemble by congenial food; but they are scarce, or not
ound beyond that distance.' Care would be needed that such local and
ly temporary populations do not upset a picture dependent on recruit-
rom the south-west.

CORROBORATION

Mr J. Taylor and Mr L. Cunnington, skipper and mate of M.F.V. *Jag* later reported that between 25 June and 3 July 1947 near the Lizard they to an exceptionally large haul of *Capros*. The gear for heaving the cod-end board was pulled down several times. The trawl in use was brand ne otherwise it would not have stood up to the great weight without splittin Buried amongst the *Capros* and scarcely visible were twenty-two baskets hake.

After 1 May the only winds as strong as Beaufort Force 6–7 occurred 4 and 6 June (Table III) and these were westerly. In La Machoire du Su and in this canyon only, could these winds have created the considerab submarine eagre on 7 June believed to have introduced the dense shoal

TABLE III. STRONG WINDS IN THE CELTIC SEA, MAY–JUNE 1947

		Scilly Observatory Wind observed		Position 48° 30′ N., 9°–10° W., Estimated wind*	
Date	Hours (G.M.T)	Direction	Force Beaufort	Direction	Force Beaufort
4 June	18	W.N.W.	6	W.'N.	6
6 June	00	W.'S.	6	W.	6
	06	W.	7	W.N.W.	6 or 7
	12	W.'N.	6	W.	6
	18	W.S.W.	5	W.S.W.	4
7 June	00	S.W.'S.	4	W.S.W.	4

* Assessed from observations by British Observing Ships in the area between 45° a 50° N., and between 7° and 13° W., supplied by the Marine Superintendent, Meteorologi Office, Harrow.

Capros. The shoal would have been carried by the current about 200 mi in 18–26 days at an average speed of 7–11 miles a day. The evidence scarcely strong enough to assert that the associated hake, a slope fish, ca from the same source.

There appears to be a neighbouring canyon, La Machoire du Nord, whi for much of its length runs west-south-west but in its upper and shallov course it turns for 5 miles to face south by west. Creation of eagres there westerly winds is less likely.

We now have two events to support the real existence of submar eagres, and both times La Machoire du Sud canyon is indicated as the si Of necessity the argument has had to be based on the coincidence of la catches of *Capros* with the occurrence a few weeks earlier over the continen slope of westerly or south-westerly winds of Beaufort Force 6–7. These prevailing winds so that the coincidences may be due to pure chance. Wher one failure to find a fit will kill the hypothesis, certainty will come only accumulating many such coincidences. The author would therefore appreci news from fishing skippers of the time, position and quantity of all catc *pros* (zulus) exceeding, say, 1000, together with information as to the sed and the duration of the haul.

dence for the hypothesis of submarine eagres could be sought by ng at the right time on the fine sandy bottom which exists at 48° 23′ N., W., inshore of the heading of La Machoire du Sud. Some hours after winds (Beaufort Force 6–7) from between south-west and west have down, *Capros* in very large numbers may there be introduced by an from the canyon.

writer is indebted to Mr A. D. Mattacola, who drew attention to the le hauls of *Capros* and has helped in many ways and to Lieut.-Cmdr. C. A. less, D.S.C., R.N.R., for his exact records of catches.

SUMMARY

le catches of boar fish (*Capros aper*) were taken near the Eddystone on tober and in early November 1951. From what is known of the ution of boar fish and from the hypothesis on the genesis of ‘submarine ’ in canyons on the continental slope, a further hypothesis has been d to explain this occurrence. It is suggested that strong west to south-rly winds on 24 September created conditions to produce a submarine the following day, and that this swept a shoal of boar fish from the -coral encrusted walls of a submarine canyon in the southern edge of ltic Sea on to the continental shelf. Thence during quiet weather the was carried by currents to the neighbourhood of the Eddystone at about a day. Further deductions which lead to means of checking the present heses have been drawn.

exceedingly large catch of *Capros* taken by M.F.V. *Jago* in the summer 7 is attributed to a shoal ejected on 7 June in a submarine eagre from canyon at 48° 22′ N., 9° 30′ W.

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ABSTRACTS OF MEMOIRS

RDING WORK DONE AT THE PLYMOUTH LABORATORY

EGG AND LARVAL STAGES OF *NYCTIPHANES SIMPLEX*, A EUPHAUSIID CRUSTACEAN FROM CALIFORNIA

By **Brian P. Boden**

Proc. Zool. Soc. Lond., Vol. 121, 1951, pp. 515–27

emale of *Nyctiphanes simplex* carries her eggs in an egg-sac. The eggs
in the nauplius stage and remain in the egg-sac throughout that stage.
arva emerges from the sac as a pseudo-metanauplius and moults im-
tely into the metanauplius stage. This is followed by three calyptopis
and the furcilia stages. There are probably nineteen types of furcilia
. Since six of these are numerically dominant, it is probable that the
'jump' stages in the furcilia series. The six dominant types are described
six furcilia stages. A key to the furcilia stages is included. B.P.B.

ICRO-DETERMINATION OF NITROGEN IN ORGANIC MATTER WITHOUT DISTILLATION

By **H. W. Harvey**

The Analyst, Vol. 76, 1951, pp. 657–60

required to determine the quantity of organic nitrogen in small
ities of unicellular algae, which can be separated from suspension by
fugation, and to complete six to eight analyses within 3 hr.

method is described by which 5–30 µg. quantities of nitrogen are esti-
in test or centrifuge tubes without distillation. After digestion with
uric acid and hydrogen peroxide, in presence of a mercury catalyst, the
ing ammonium sulphate is treated with an excess of hypobromite. The
ual hypobromite is determined by titrating the iodine, liberated on
on of potassium iodide and acetic acid, with standard sodium thio-
ate solution.

order to test the accuracy of the method, analyses of acetanilide were
and yielded the following results:

Nitrogen taken (µg.)	Nitrogen found
10·32	10·1, 10·2
20·65	19·2, 20·6
41·3	40·7, 41·4
20·65	20·7 as mean of four analyses.

H.W.H.

The Swimbladder and the Vertical Movements of Teleostean Fishes. I. Physical factors

By **F. R. Harden Jones**

Journ. Exp. Biol., Vol. 28, 1951, pp. 553–66.

The vertical movements of a teleostean fish may be restricted by the presen of the swimbladder which will increase or decrease in volume when the fis moves up or down in the water. The restriction that the swimbladder impos to vertical movements involving a reduction in pressure will depend physical factors such as: (1) the resistance that the bladder and body-wa offer to the expansion of the bladder gas; (2) the percentage volume of th swimbladder and the density change of the fish when it is subjected a reduction in pressure; (3) the pressure reduction that leads to the ruptu of the bladder wall.

Various experiments were made on the perch, *Perca fluviatilis*, the wrass *Crenilabrus melops*, the rockling, *Onos mustela*, and the dragonet, *Callionym lyra*. The results showed that there was a relation between the relative size the swimbladder and the change in the density of a fish when it was subject to a pressure reduction; that the bladder and body-wall of the perch off little resistance to the expansion of the bladder gas; and that the danger of t bladder wall rupturing might restrict the extent of rapid movements made the perch. F.R.H.

A Larval Hoplophorid (Crustacea) from Bermuda

By **Marie V. Lebour**

Proc. Zool. Soc. Lond., Vol. 121, 1952, pp. 753–7

An interesting larva is described belonging, probably, to the genus *Notostom* No larva of this genus is so far known and it is probable that the usual habit is in deep water. The suggestion is offered that the normal larva is small a thin-skinned and that the present large form which was found at a less depth may have been delayed in metamorphosing or not been able to met morphose at all owing to the uncongenial surroundings. M.V.

The Induction of Regeneration in the Hydroid *Cordylophora lacustris*

By **Janet Moore**

Journ. Exp. Biol., Vol. 29, 1952, pp. 72–93

Reconstitution masses of the hydroid *Cordylophora lacustris*, made chopping up unspecialized tissue and piling the fragments into heaps, ma regenerate hydranths 'spontaneously'. An oral cone grafted into a ma

ces, at the point of grafting, the development from mass tissue of hydranth
ns basal to the oral cone. The induced hydranth develops at an accelerated
and spontaneous regeneration at other sites is inhibited. Beadle and
's report that oral cone grafts induce hydranth development in *Cordylo-*
reconstitution masses is confirmed and extended.

e inducing properties of oral cone grafts are shared by grafts of other
ns of the hydranth (tentacular ring, subtentacular region, hydranth neck,
even small fragments of tentacle) and by the rudiments of developing
anths (tips of outgrowths from masses) but not by tissue lacking hydranth
entiation (stem coenosarc and stolon tips). Induction may be produced
ifferentiated tissue grafts when inverted or fragmentary, but not when
rated or killed, nor has induction occurred with thin agar or cigarette
r barriers inserted between the graft and host. There is no evidence that
anth induction is mediated by a diffusing chemical; direct close contact
living differentiated tissue is necessary for induction to be produced in
ecialized tissue. J.M.

AUTONOMIC NERVOUS SYSTEMS IN LOWER CHORDATES

By J. A. Colin Nicol

Biol. Rev., Vol. 27, 1952, pp. 1–48

review presents a detailed survey of the visceral efferent systems of lower
iates from the protochordates to the Amphibia. The classical system of
ley's is used as a basis for discussion, a morphological description of the
m in each of the major groups is presented, and this is followed by
tional analyses, as far as the data permit. In these lower forms it is possible
ace an elaboration of complexity in the autonomic systems from the
ric nerve net of balanoglossids to the differentiated amphibian organiza-
characteristic of tetrapods. The system is rudimentary in cyclostomes,
chromaffin tissue is present, and the vagus supplies the gut and heart.
pathetic and parasympathetic systems show great diversity and specializa-
in fishes, from a rather primitive arrangement in elasmobranchs, to a high
ee of differentiation in teleosts. The latter are characterized by a greater
larity in organization of the sympathetic trunks, and by augmentation of
ields of sympathetic innervation in the head and other peripheral effectors.
e Dipnoi the autonomic system appears to be on a lower level of morpho-
al organization than in teleosts. In the Anura it is organized in the
ern found in mammals, and has cranial and sacral parasympathetic, and
ominal sympathetic components. A progressive elaboration of physio-
al complexity is traced along with morphological differentiation in the
r chordates, and the implications discussed. Analysis of evidence leads

to the conclusion that the autonomic nervous systems of vertebrates show tv main independent lines of evolution from some simple level of organizati such as that found in extant elasmobranchs. These two lines occur in actin pterygians leading to modern teleosts, and in choanichthyes leading to Dipn and tetrapods, and there has been a considerable degree of independe specialization and differentiation in these two groups. J.A.C.

UNTERSUCHUNGEN ÜBER DIE ALGENBEWOHNENDE MIKROFAUNA MARIN HARTBÖDEN. I. ZUR OEKOLOGIE UND SYSTEMATIK DER NEMATODENFAU VON PLYMOUTH

(ON THE ECOLOGY AND TAXONOMY OF THE FREE-LIVING MARINE NEMATODES OF PLYMOUTH)

By W. Wieser

Österr. Zool. Zeitschrift, Bd. 3, 1951, pp. 425–80

The free-living nematodes inhabiting the following seaweeds were studie *Ceramium* sp., *Cladophora rupestris*, *Lomentaria articulata* between +3· and +0·8 m., *Gelidium corneum* between +2·75 and +1·10 m., *Fucus serrat* between +2·00 and +0·20 m., *Gigartina stellata* between +2·00 and +1·00 m *Porphyra laciniata* between 1·95+ and +1·50 m. (above C.D.) and *Nitophyllu punctatum* between −0·70 and −3·00 m. (below C.D.). All samples we taken in the Tinside area of Plymouth Sound.

The population of the small and procumbent *Gelidium corneum* proved to b richer both in numbers and in species than that of the tuft-like *Ceramium* s The composition of the two faunas differs considerably, the former bei dominated by relatively long and detritophilous species, the latter by sma ones of the family Chromadoridae.

The composition of the nematode fauna of the two algae *Gigartina stella* and *Fucus serratus* depends largely upon the extent to which epigrowth developed on them. Apart from that, the fauna of *Gigartina stellata* proved be much richer than that of *Fucus serratus*, which is due to the dens branching of the former weed. The habitats richest in species appear to b *Gigartina stellata* and *Nitophyllum punctatum*.

The distribution of length-classes in a given nematode population depen upon the shape of the seaweed inhabited. Two modes of distribution length-classes were distinguished, viz. one with the smallest forms betwee 0·4 and 1 mm., amounting to more than 70 % of the total population, an a second one with these smallest forms comprising not more than 35 % of th population and being outnumbered by specimens over 1·5 mm. The vertic distribution of all species found is given. Typical species for the highest an

t zone, respectively, can be indicated with a high degree of probability.
ether seventy species have been listed of which three genera, eleven
s and one variety are new to science. These are described in detail.

W.W.

NFLUENCE OF THE NATURE OF THE SUBSTRATUM ON THE METAMORPHOSIS F THE LARVAE OF MARINE ANIMALS, ESPECIALLY THE LARVAE OF *OPHELIA ICORNIS* SAVIGNY

By D. P. Wilson

Ann. Inst. Océan., T. 27, 1952, pp. 49–156

vey of the literature concerning larval settlement shows that the larvae
umber of species are able to postpone metamorphosis for a period of
during which they are able to select the substratum in or on which to
morphose. This ability is most strongly developed in species confined
ngle type of bottom deposit. Such a species is *Ophelia bicornis* Savigny
larvae are unusually suitable material for experimentation. In a large
er of experiments they have been used in an endeavour to determine the
s by which the larvae distinguish one sand from another. It is concluded
ertain sands are repellent by virtue of organic matter, or living micro-
isms, on the surface of the grains, and that provided the sand be not
able in grade the larvae favour relatively clean sands, the cleanest sands
lly found in nature apparently containing sufficient nourishment for the
. Organic materials present on such grains may be also of a different
from those on grains which are repellent.

paper contains discussions of various matters relative to the main
. It is shown that *Ophelia bicornis* is found only in a few restricted
ies from the Bay of Biscay to the entrance to the English Channel, and
l localities are bays or estuaries where strong tidal scour may occur and
nd clean, coarse and loose. Certain physical features of sands, parti-
y the readiness with which their grains sink or float when sprinkled dry
water, are investigated.

D.P.W.

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1951–52

Council has to report with deep regret the death of the Librarian of the outh laboratory, Miss M. A. F. Sexton, who had served the Association y and devotedly for many years.

e following have been elected Vice-Presidents of the Association during ear: Major E. G. Christie-Miller and Mr Morley H. Neale, C.B.E.

Council and Officers

ur ordinary meetings of Council were held during the year, three in the s of the Royal Society, and one at the Plymouth laboratory. At these the ge attendance was eighteen.

pecial meeting of Council was held in the rooms of the Royal Society October 1951 to consider the pay of the crews of the Plymouth research

ring the year Dr C. F. A. Pantin, F.R.S., was appointed as Governor enting Cambridge University in place of Prof. J. E. Smith.

Edward Hindle, F.R.S., attended a Royal Garden Party at Buckingham e as a representative of the Association in July 1951.

Plymouth Laboratory

ring the year the waterproof covering to the roof of the western half north building has been stripped and the roof has been relaid with lt. The covering to the roof of the bridge and passage connecting the and south buildings has also been renewed with a roofing felt.

small laboratory has been built, adjoining the constant temperature ng, to be used for research with radioactive substances. The furnishing tting out of this laboratory has also been completed.

mission has been obtained from H.M. Treasury to have detailed plans pecifications prepared for the extension of the library building.

Aquarium

e aquarium has maintained its popularity with the general public and ontinued to play a part in the education of large numbers of school ren. Tanks have been well stocked, and among unusual exhibits may be ioned the Red Bandfish (*Cepola rubescens*) and the very rare Stone Bass reck-fish (*Polyprion americanus*), a specimen of which was caught by men off Hope Cove, near Salcombe.

Some improvement to visibility in the tanks on the south side has bee effected by fitting ground glass to the front windows, thus reducing afternoo sunbeam dazzle.

Research Ships

Apart from periods for annual refit the research vessels *Sabella*, *Sula* an *Gammarus* have worked regularly throughout the year. During the year th face-plate on the stern-post of the *Sabella* has been renewed, and a new rudde fitted to the *Gammarus*. Alterations have been made to the engine room of th *Sula* to give additional clearance above the engine.

New Research Ship

The Council has pleasure in reporting that during the year H.M. Treasur has sanctioned a grant to meet the cost of building a new research ship. Thi will be the first time that the Association has been able to build a ship speciall designed for its research requirements.

The ship is to be steel-built, 115 ft. B.P., and powered by a main dies engine, with an auxiliary for slow speeds. Messrs Graham & Woolnough Liverpool are acting as naval architects to the Association, and are designin the ship to incorporate requirements and details which have been mainl drawn up by Mr G. A. Steven. The ship is to be built in the yard Messrs Philip and Son Ltd. at Dartmouth.

The Staff

Dr Mary Parke and Mr P. G. Corbin have been promoted to the grade Principal Scientific Officer.

Mr D. B. Carlisle and Mr G. R. Forster were appointed to the staff of th Plymouth laboratory in October 1951, in the grade of Scientific Officer.

Dr W. R. G. Atkins, O.B.E. (Mil.), F.R.S., was appointed a Command of the Order of the British Empire in the King's Birthday Honours.

Dr D. P. Wilson has been awarded the Prix Georges Kohn 1951 of th Institut Océanographique, Paris.

Dr L. H. N. Cooper attended the Ninth General Assembly of the Inte national Union of Geodesy and Geophysics in Brussels in August 1951, an the thirty-ninth meeting of the International Council for the Exploratio of the Sea in Amsterdam in October 1951.

Occupation of Tables

The following one hundred and five workers have occupied tables at th Plymouth laboratory during the year:

E. Adams, Plymouth (Library).
Dr Daphne Atkins, London (Biology of *Pinnotheres*).
Dr and Mrs G. Bacci, Naples (Sex reversal in *Ophryotrocha*).
R. Bainbridge, Oxford (Plankton interrelationships).

D. BALLANTINE, Development Commission (Taxonomy and culture of marine Flagellates).
G. BEST, London (General).
NNA M. BIDDER, Cambridge (Digestion in Cephalopods).
R. BIEBL, Vienna (Algal physiology).
RKETT, Lowestoft (Bottom sampling).
. BLASCHKO, Oxford (Amine oxidase in *Octopus* liver).
P. BODEN, Cape Town (Phytoplankton and Euphausians).
BOYCOTT, Oxford (Brain and behaviour of *Octopus*).
BRIGHTWELL, Peacehaven (Experiments with *Eupagurus*).
M. BROWN, London (Parasitic Dinoflagellates).
DITH BULBRING, Oxford (Amine oxidase in *Octopus* liver).
RSA, Kenton (Dinoflagellates).
CANNY, Cambridge (Algae).
CARLISLE, Oxford (Endocrinology of Ascidians and *Leander*).
. S. CARTER, Cambridge (Library).
CHAPPELL, Penzance (Library).
CHILDS, Plymouth (Gall bladders of fishes).
. N. J. CHIPPERFIELD, Brixham (Library).
CHRISTIE, London (General).
CLARK, Glasgow (Swimming in Polychaetes).
. L. CLARKE, Woods Hole (General).
M. COLLIS, London (*Sabellaria*).
. COOK, Cambridge (General).
OPLEY, Kenya (General).
COSWAY, Torquay (Library).
. COWPER, Reading and C.S.I.R.O., Australia (Fisheries methods).
. J. CRISP, Brixham and Bangor (Library).
CURRIE, National Institute of Oceanography (Oceanography).
ELGOOD, Nigeria (Library).
THERINGTON, London (Sporozoa in marine annelids).
ARIA FELINSKA, Cheltenham (Ciliates).
. FISHER, Reading (Vitamin A in marine animals).
. FORSTER, D.S.I.R. (Biology of prawns).
R. H. C. F. FRAMPTON, R.N. (Rtd.), Plymouth (Library).
ERA FRETTER, London (Molluscs).
. GODBEY, Cardiff (Architecture of Marine Stations).
ANSEN, Copenhagen (Library).
. J. HART, National Institute of Oceanography (Plankton).
. HASWELL, London (Library).
. N. HILL, Cambridge (Geology of the English Channel).
. HOLMES, Oxford (Interrenal and adrenal bodies).
. HORRIDGE, Cambridge (Annelid nervous systems).
Cdr (D) D. C. HOWE, R.N., Plymouth (Library).
OYLE, London (Neuromotor activity of *Phallusia*).
. HUNT, Newton Ferrers (Fouling organisms).
HUTTON, Miami (Fulbright Scholar) (Parasitology).
J. JACKMAN, Paignton (Library).
. G. JONES, Brixham (Library).
. JONES, Cambridge (*Leucosolenia*).
. JONES, Aberystwyth (Ecology of *Gracilaria*).

Dr Elizabeth M. Kampa, Cambridge (Retinal pigment in fish).
R. S. Keir, Development Commission (Development of Lamellibranchs).
G. Kerkut, Cambridge (Nervous systems of *Astropecten*).
Miss J. Kerslake, London (Ectocarpaceae).
Prof. W. B. R. King, F.R.S., Cambridge (Geology of the English Channel).
Major B. G. Kinloch, Uganda (General).
K. Kolstad, Oslo (Ecology of *Patella* species).
M. E. Korn, London (Cucumarians).
Miss P. Kott, Australia (Biology of Tunicates).
Dr Marie V. Lebour, Cawsand (Decapod Crustaceans).
Prof O. E. Lowenstein, Glasgow (Electrophysiology of elasmobranch labyrinth).
Dr A. G. Lowndes, Wells (Entomostraca).
M. F. Mahmoud, Manchester (*Scalpellum* and *Balanus*).
Dr Sheina M. Marshall, Millport (Egg laying in *Calanus* and use of isotopes).
A. J. Matty, Nottingham (Elasmobranch endocrinology).
P. A. Mayes, London (Osmotic relations of *Littorina* species).
R. Mayne, Plymouth (Library).
P. B. McFarlane, Cambridge (Geology of the English Channel).
Prof. N. Millott, Jamaica (Melanin formation in invertebrates).
Miss M. Morris, Australia (Diurnal rhythms in luminescence).
J. E. Morton, London (Marine Pulmonates).
B. S. Newell, Colonial Fisheries (Analysis of sea water and oceanographic methods)
Miss L. M. Newton, British Museum (Natural History) (Algae).
O. Nordli, Florø, Norway (Phytoplankton).
Dr W. Ohle, Germany (Chemistry of sea water).
Dr A. P. Orr, Millport (Egg laying in *Calanus* and use of isotopes).
Prof. J. H. Orton, F.R.S., Liverpool (*Patella*).
A. H. Papworth, Northampton (General).
Miss G. D. Parry, Cambridge (Ionic regulation in prawns).
Prof. E. Percival, New Zealand (General).
Prof. J. E. G. Raymont, Southampton (General).
Miss B. Rickard, Plymouth (Library).
T. R. Roberts, Plymouth (Library).
G. A. Robinson, Development Commission (Phytoplankton).
D. J. Rochford, Australia (General).
J. B. Rogers, Admiralty (Oceanographic apparatus).
L. Sömme, Oslo (Culturing of *Ulva*).
Dr A. J. Southward, Port Erin (Intertidal ecology).
B. W. Sparrow, Newton Ferrers (Fouling organisms).
Dr C. P. Spencer, Bangor (Physiology of diatoms).
Miss F. A. Stanbury, Plymouth (*Cladophora*).
Dr M. F. Sutton, London (Regeneration in *Ciona*).
H. Tebble, British Museum (Natural History) (Polychaetes).
Miss M. Treharne, Plymouth (Library).
Miss B. C. Upton, London (Methylene blue staining in Polychaetes).
B. Watts, Watford (General).
Dr G. P. Wells, London (*Arenicola*).
G. L. Wilkins, British Museum (Natural History) (Molluscs).
R. F. Wrighton, Birmingham (General).
H. V. Wyatt, Plymouth (Littoral ecology).

ring the year there have been a large number of short visits made by
sts to discuss problems with members of the staff, or see the work of the
tory. Among these the following research workers from overseas have
visits: B. Ulrich, Düsseldorf; Dr and Mrs Gorbman, Columbia
rsity; Dr C. S. Piggott, U.S. Embassy; Dr R. S. Dietz, U.S.N. Hydro-
ic Department; Dr Vera Koehring, New York; J. Lyman and T. S.
n, U.S.N. Hydrographic Department; L. B. Mendel, Cape Town;
orthington, D. J. Owen, J. Hahn and C. Iselin, Jr., Woods Hole;
von Brandt, Hamburg; Prof. Hans Brattström, Bergen; Prof. K. Hidaka,
o; Miss H. I. Jørgensen, Copenhagen; Dr L. R. Donaldson, Seattle.
e U.S. Research Ship *Albatross III* visited Plymouth during September
working from Woods Hole under the scientific leadership of Mr V.
hington.
e Easter Vacation Courses were conducted by Mr G. M. Spooner and
. G. Corbin, and were attended by 42 students from the following
rsities and University Colleges: Oxford, Cambridge, London,
ngham, Liverpool, Leeds, Sheffield, Belfast, Nottingham, Newcastle,
ampton, Aberystwyth, Bangor and Cardiff.
o during the Easter Vacation Mr K. G. Messenger and Mr I. G. StC.
e brought seven boys from Uppingham School and Mrs M. E. Allen
from the Bec School, London.

ific Work of the Plymouth Laboratory Staff

Physics and Chemistry of Sea Water

urvey has been made of the surface temperatures and the mean tem-
re of the water column at Station E 1 for each year since 1921 to 1949,
included, but with a war gap. The maximum column-temperature
C. was in 1949, followed by 15·43° C. in 1921, and the maximum sun-
was also in 1949, but there was no obvious relation between hours of
ine, or vertical illumination, and water temperature in the other years.
force and evaporation are important factors difficult to assess, but
. R. G. Atkins and Miss P. G. Jenkins have, as a first step, tabulated the
ence between the mean monthly vapour pressure of the air, as given by
eteorological Office and the vapour pressure of the surface water of the
he excess pressure of the sea is extremely irregular, but is usually least
mmer, which suggests that cooling by evaporation is then at a minimum.
e daylight records for 1947–9 are being published by the Royal Meteoro-
l Society, who have also recently published measurements of the
ent transmission of light through clouds, made by Dr Atkins during the
using observations on cloud thickness and height obtained by the
rs of the Meteorological Flight of the Royal Air Force at Bircham
on in Norfolk. The relevant daylight charts were got using a sodium cell

no longer on the market; tests are accordingly being made on the stability o another emission cell as probably preferable to the selenium rectifier cel which has, even in England, only a quite limited period of stability.

The work on the scattering of light in natural waters and its variation wit angle of incidence has now been prepared for publication by Dr H. H. Pool and Dr Atkins; equipment has been assembled for following this variatio through the spectrum.

Dr L. H. N. Cooper has continued his examination of hydrographica records from the Celtic Sea collected during the last 40 years and has publishe three papers in Volume xxx, No. 3 of the *Journal*.

In one he has described the physical and chemical oceanography of th water bathing the continental slope of the Celtic Sea. The extension of th Gulf of Gibraltar water into the Eastern North Atlantic is very variable bot in space and time and, at a given position, very large changes in temperatur and salinity may occur in a few months. South-west of Ireland at aroun 800–1000 m. there is a fluctuating conflict between this Gulf of Gibralta water deficient in oxygen (65–68% saturated) and well oxygenated (>85% cascaded water from the north, probably from the Rockall Table Mount Between 1000 and 2000 m. depth south of the Celtic Sea a decrease i phosphate content of at least 0·2 μg.-atom/l. seems to have occurred betwee 1930 and 1950.

The properties have been outlined of a number of recognizably differen water masses in the ocean near the continental slope. The vertical structur of these waters in June 1914 has been derived from observations by th Norwegian vessel *Armauer Hansen*. The deep water observations made b R.R.S. *William Scoresby* and R.R.S. *Discovery II* on behalf of the Plymout laboratory have been worked up.

In the second paper Dr Cooper has described his hypothesis of 'capsizing of water masses over a continental slope in high on-slope winds. Such process would be powerful enough to produce the observed distribution o properties in the ocean abreast of the slope. Although it is compatible with th observations available here, much more work is needed to prove it. Attentio has been focused on the importance of the topography of the continent slope in studies of the exchange of water masses and their nutrient resource between the deep ocean and the continental shelf. On the basis of the distribu tion of temperature and salinity, the existence of a submarine canyon in th continental slope was predicted at 50° 30′ N., 11° 00′ W. This canyon ha been sought by H.M. Survey Ship *Cook* and found at the position forecas The success of this prediction will now allow the publication of other wor on water movements over the slope, which has had to be held up for con firmation of certain debatable points.

The silicate analyses obtained in 1950 have allowed the preparation a paper in which the chemistry of silicate in sea water has been re-examine

December 1950 a hydrographical investigation was made of water move- along the South Devon coast in connexion with the occurrence of g off Plymouth.

monthly cruises to Station E 1 and routine determinations of phosphate, phosphorus and silicate have been continued by Mr F. A. J. Armstrong. les taken by Dr Cooper on two short cruises in December 1950 have een analysed. In June and July 1951, samples were taken in the Teign y and analysed to assist Prof. J. H. Orton in his studies on *Patella*.

thods of determining and analysing the suspended matter in smaller es have been developed, and it has been found practicable to use samples out 2 litres, which may be collected with a Nansen-Pettersen bottle. nal changes at Station E 1 are being studied by these methods.

Plankton

mations of the chlorophyll content of water at Station E 1 have been ed by Dr Atkins. Using the Unicam spectrophotometer it is possible a good spectral absorption curve from as little as one litre of surface p water even in late autumn.

H. W. Harvey, in collaboration with Dr C. P. Spencer of the Bangor e Biological Laboratory, has continued investigating the uptake of en compounds by *Nitzschia closterium*, the formation of plant pigments, e rate of growth.

monia nitrogen is utilized and built up into organic nitrogen by nitrogen nt cells as rapidly in darkness as in the light, nitrate nitrogen less y, and nitrite nitrogen very slowly or not at all, whereas it is utilized idly as ammonia or nitrate nitrogen in the light. This suggests that is not reduced via nitrite in the plant in darkness, the nitrite stage in duction of nitrate to ammonia being by-passed. An account of a rapid d of determining microgram quantities of organic nitrogen in plant and l tissue has been published in the *Analyst*.

synthesis of chlorophyll *a* and other pigments by *Nitzschia* stops the growth of a culture where the available nitrogen in the medium is sted or nearly exhausted, while cell division continues with the forma- f nitrogen and chlorophyll deficient cells. When available nitrogen is ed to these deficient cells, synthesis of chlorophyll can take place in ss. The quantity of chlorophyll and other pigments per cell is greater oms grown in dim light than in bright light, that is, at light intensities rable to those at depths of 30 m. and of 10 m. respectively in the open a bright day with the sun obscured by cloud. Preliminary experiments that the effect of changing the light intensity during the exponential h of the diatom is complex.

ch time has been devoted to improving technique for estimating the se in number of diatoms (mean time per division) during growth in bacteria-free culture under conditions of constant light, temperature and aeration. Dr Spencer's observations, some of a preliminary nature, are showing the following trends. Provided the diatom has become adapted to the environment, growth proceeds at an exponential rate, which is remarkably constant from experiment to experiment, until the limiting nutrient in the medium is exhausted; then the rate gradually decreases until a stationary stage is reached. This exponential rate is the same whether the diatoms are supplied with ammonium, nitrite or nitrate, and is the same over a wide range of hydrogen ion and bicarbonate concentrations, and is similar over a wide range of light intensities.

When the diatoms have not become adapted to the environment, th exponential rate tends either to be irregular, or, as in many experiments, t change suddenly to a lesser exponential rate which remains constant unt one or other of the inorganic requirements are exhausted in the medium.

Preliminary experiments show that periods of darkness during the growt of the culture have no effect on the rate of cell division during the illuminate periods, provided the supply of carbon dioxide is not limiting.

When a medium is inoculated with the diatoms there is a lag before ce division starts. This lag period is constant from experiment to experimen when the inoculum has been growing exponentially in a medium which is no deficient in a nutrient. The period is less when the diatoms are subculture into a medium containing ammonia, than when they are subcultured int one containing nitrate or nitrite as nitrogen source. When the inoculu is taken from a culture in which the nitrogen source has become exhauste that is, consisting of cells which are nitrogen deficient, the lag period lengthened progressively with the nitrogen deficiency of the cells. The lengt of the lag period rises until the cells of the inoculum have reached the poi of maximum deficiency and thereafter remains constant for a period of som days. Further increase in the length of the lag period occurs only aft storage of the inoculum cells for considerable periods. During the lag peric the cells synthesize both organic nitrogen and chlorophyll.

It is the final aim of this investigation to relate culture experiments to th growth of phytoplankton in the sea; their adaptation to varying light intensity and temperature, and the effect upon them of those traces of unknow substances which render waters from different localities fertile or infertile the growth of particular species.

Over 100 different plant organisms, mainly flagellate forms, are now bei maintained by Dr Mary Parke in species-pure cultures in addition to ma mixed cultures containing organisms of interest awaiting isolation. A stu of some new members of the Chrysophyceae with three flagella is in progre During the year cultures for research purposes have been sent successfully air to U.S.A., Norway, Italy and France. A large number of cultures ha also been sent to institutions in this country.

s D. Ballantine, on a Development Commission Fisheries Research ing Grant, has been studying the effects of different salinities and of ial sea water on cultures from the stocks maintained by Dr Parke. Over -five different forms have been used, and although these have been ained for over six months very little difference beyond the normal on within a species has been found. The density of the organisms in ltures did, however, vary considerably.

s Ballantine is also making a comparative study of the methods of tion of numbers of flagellates in a unit volume of water, and preparing ptions of three new species of dinoflagellates.

F. S. Russell has re-examined a number of samples of the copepod *us* from the collections on which his earlier observations on the vertical bution and biology of the species have been based. It can be stated that blished results refer to the form *helgolandicus*, the more northerly form *chicus* having occurred only in small numbers in the catches. A short nt of this revision has been published in Volume xxx, No. 2 of the *Journal*.

Russell has also succeeded in rearing to maturity some specimens of re medusa *Gonionemus*. These were kindly sent, while being budded off he hydroid, by Dr H. O. Bull, who found them in the tanks of the Dove e Laboratory, Cullercoats.

P. G. Corbin's inspection of the 1951 weekly half-hour oblique hauls ne 2 m. stramin ring-trawl shows no major change in the macroplankton ction of this area from the low level prevailing during recent years. The for 1949 was published in Volume xxx, No. 2 of the *Journal*.

Fauna and Flora of the Sea Floor

ing been invited by the Comité de Perfectionnement, Institut Océanoique, Paris, to submit for the Prix Georges Kohn 1951 a paper on the nce of the nature of the substratum on the metamorphosis of the of marine animals, Dr D. P. Wilson has written a memoir in which is ded an historical survey of the literature relating to this subject. Much memoir is, however, based on his work with the larvae of *Ophelia is* and contains a long and detailed account of three seasons' experil work with this species. The geographical distribution of *Ophelia is* and its local distribution in the Exe estuary is considered in relation factors, so far as they are known, which influence settlement. Observaon the wettability of some sands are also included. The memoir has published in the *Annales de l'Institut Océanographique*.

ce this memoir was completed a further series of experiments with the of *Ophelia bicornis* have been made. These have yielded results which pected to throw further light on the main problem when they have been lly studied.

G. M. Spooner has continued his studies on amphipod crustaceans.

During a visit to the British Museum the greater part of the *Gammarus* material from British, N. European, and Arctic waters was examined and much of it re-identified. Following the publishing of a paper on *G. zaddachi oceanicus*, another dealing with the two other arctic forms, *G. setosus* Dementiera and *G. wilkitzkii* Birula, is in preparation. Both of these are valid species. Overlooked characters have to be pointed out. The latter species is more different from the temperate *G. zaddachi* than has been supposed.

An identification key for British species of *Gammarus* has been prepared.

The amphipod faunas of the trawling grounds, where these animals form an important element in the diet of bottom-feeding fish, are receiving attention. In addition to the detailed recording of amphipods in quantitative bottom samples and in the stomachs of young rays, a regular examination has been made of the fauna lodging amongst coils of *Chaetopterus* tubes and on the carapaces of spider crabs.

A watch has been kept by Mr Spooner on the westward spread of the mollusc *Crepidula fornicata* (American slipper limpet) into the Plymouth area, and of the grass *Spartina townsendii*, which is now well established in the estuaries of the Avon, Erme, Tavy, Tamar, and Lynher rivers.

A re-examination of the species of sessile Scyphomedusae (Lucernariidae) in the Plymouth area has been started by Mr P. G. Corbin. From preliminary work it appears that the existing list is in need of revision.

Dr Mary Parke's revision of the late Mr G. F. Tregelles's manuscript on the marine algae of Devon has now been completed and is in the press (being published as an appendix to the *Transactions of the Devonshire Association*).

In addition Dr Parke has compiled a preliminary Check List of the British marine algae which includes the plant divisions Cyanophyta, Chlorophyta (Chlorophyceae), Chrysophyta (Xanthophyceae), Phaeophyta and Rhodophyta. Miss D. Ballantine has assisted Dr Parke with some of the work involved in the production of the Check List.

Dr H. G. Vevers has continued to use his underwater photographic apparatus in a quantitative survey of the epifauna on the trawling and dredging grounds in the Plymouth area. He has published a paper in Volume xxx No. 1 of the *Journal*, which describes the apparatus and its mode of operation and gives the first results obtained. It has been found that the dense aggregations of *Ophiothrix fragilis* photographed on some grounds are not of seasona occurrence, but represent constant populations, which can be found at any time of the year. Further work is being done on the biology of these populations, in which the average density of the brittle-stars may be more than 100 individuals per square metre in certain places. A start has also been made on a photographic survey of the rich rocky bottom area near the Mewstone. Further improvements are being made in the design of th photographic apparatus.

In his work on the pigmentation of marine animals Dr Vevers has completed eliminary study of the carotenoids in the integument of dark brown imens of *Asterias rubens*, published in Volume xxx, No. 3 of the *Journal*, s now working on the pigmentation of other colour phases of this species. he dark brown specimens the main pigment is esterified astaxanthin, of which is present in the form of a carotenoid-protein complex.

r N. A. Holme has been continuing work on the 'biomass' of the bottom a in the Plymouth area. Material collected at twenty stations during has been examined, particular attention being paid to possible sources rror in the collection and subsequent treatment of samples. A sample ring $\frac{1}{2}$ m.^{2} is sufficient for a qualitative evaluation of the fauna, but few ies are sufficiently dense for an adequate estimation of their numbers in mple of this size. The total figures for the quantity of living matter are e same order (*c.* 50 g. fresh weight per m.^{2}) at each station, so that the lts will enable long-term changes in density to be followed. Several ies, previously common, are now absent or rare; this may be correlated the hydrographic changes which occurred in the early thirties. A new rd for the area is of a species of *Glossobalanus* (Enteropneusta), three imens being taken by the bottom-sampler in the neighbourhood of the ystone. This species probably produces the tornaria larvae which are d in the Plymouth plankton from time to time.

ollections of *Ensis*, together with the sand they inhabit, have now been on most of the suitable beaches of south Devon and Cornwall; other ples have been obtained from the Scilly Isles, Jersey, and Milford Haven. soils have been mechanically graded by sieving and rates of sedimenta- and some correlation was found between the type of soil and the distribu- of the three species. The species inhabit a fairly wide range of sands, *rcuatus* showing a marked preference for coarser soils. *E. siliqua* and *nsis* inhabit similar grades of sand, but the former is typically a shore or ow water species, while the latter is typically an offshore species. Clearly are many other factors besides soil grade affecting distribution, one e most puzzling being that of depth; differences in soils at varying depths nsufficient to explain this.

r G. R. Forster has completed his paper on the breeding and growth of *der serratus*, which was published in Volume xxx, No. 2 of the *Journal*, ther with a note on the biology of *Leander squilla*.

bservations on the behaviour of *Leander* spp. have been continued in laboratory and in the field, the chief problem being to ascertain the re of the orientation which these animals use during their tidal and diurnal ations. Both vision and current perception appear to be employed, ibly linked with an internal rhythm in activity.

he Admiralty has been kind enough to allow Mr Forster to take a course hallow water diving with the Royal Navy. With the co-operation of the ng School, H.M.S. *Defiance*, preliminary underwater observations have been made. These showed that several animals, notably *Cerianthus lloydii* an *Myxicola infundibulus* are very abundant beyond the *Laminaria* zone in th Sound, although they have very rarely been taken by dredging. It is hope to make a survey of shallow water sublittoral communities by direc observation when diving apparatus is available.

Physiology of Marine Animals

Dr J. S. Alexandrowicz has investigated tumours noticed by Mr G. A Steven in a specimen of the red mullet (*Mullus surmuletus*). These tumour proved to be a manifestation of the Lymphocystis disease regarded as resultin from infection by a virus agent. Special attention has been given to th inclusion bodies in the growing tumour cells which have been considered t represent the agglomeration of the virus substance. The transformations c these inclusion bodies observed in certain cells are interpreted as stages in th life cycle of the virus agent during which it presumably acquires the abilit to transmit the infection. The results have been published in Volume xx No. 2 of the *Journal*.

In continuation of his investigation on the muscle receptor organs i crustaceans, Dr Alexandrowicz has examined these organs in the thoraci region of *Homarus vulgaris* and *Palinurus vulgaris*. It has been found tha apart from elements belonging to the same category as those previousl reported from the abdomen, there are in the thorax nerve cells of a differen kind which are also connected with certain muscles. The results are nearl ready for publication.

The first part of the observations on the nervous system in stomatopods ha been prepared for publication. It deals with the system of median connective homologous to the so-called unpaired nerve in insects. It has been show that in *Squilla mantis* it is in relation with the large blood sinuses and contai two sorts of nerve fibres: one of these comprises motor elements; it i suggested that the other may have a neurosecretory function. An accou of this work is being published in the *Pubblicazioni della Stazione Zoologi di Napoli*.

In a series of investigations Dr J. A. C. Nicol has been able to trace th neuronal organization and pathways of certain giant nerve fibres in variou polychaete worms. These histological studies have laid a firm foundation fo carrying out physiological experiments and interpreting the results. I *Branchiomma vesiculosum* the giant axon system has been utilized to investigate the action of various pharmacological agents in the neuromuscul junction. Although essentially cholinergic it has been found that this junctio is not blocked by atropine and D-tubocurarine. It thus stands in marke contrast to the conditions encountered in hirudineans and vertebrates, i which curare has a paralyzing effect on locomotory muscles, and points t the existence of some diversity in receptor surfaces concerned with neur

ular transmission in the annelid phylum. This work has been accepted
blication in *Physiologia Comparata et Oecologia.*

Nicol has also begun a series of investigations on the physiology of
escence in marine animals, and attention has first been focused on the
haete *Chaetopterus variopedatus.* In this animal the production of light
to the secretion of a luminescent material from certain restricted regions
body, especially from glands on the aliform notopodia and in notopodia
posterior regions. The glandular elements responsible have been
ified as densely staining eosinophilic epithelial cells. By the use of re-
ng apparatus consisting of a multiplier photocell and string galvanometer
cilloscope it has been possible to obtain photographic records of the
nescent response, although the absolute intensity of the light is very low.
studies have shown that the luminescent response is under nervous
ol, and light is produced only as the result of external stimulation. The
glands, under electrical stimulation, display some of the characteristics
uscular effector organs, in that fatigue, summation, and a sustained
nse can be obtained. Additional studies have involved the action of
lanced ionic solutions and pharmacological agents on the light glands.
accounts of this work have been published in Volume xxx, No. 3 of the
al.

J. Lowy has made a detailed investigation of the mechanism of
contraction in lamellibranch muscle. The spontaneous electrical and
anical activity in the intact posterior adductor muscle of *Mytilus edulis*
orded continuously with the animal in water or exposed to air. Records
asic contraction of the muscle when the animal is in water show that the
of relaxation can be 'accelerated' and evidence from experiments with
ctor muscle preparations suggests that this may be due to the action of
itory nerves. Both phasic and tonic contraction of the muscle is accom-
ed by electrical activity and when the load supported by the muscle is
ased the frequency of electrical activity also increases. The results
ined in these experiments lead to the conclusion that the *Mytilus* muscle
ot maintain a steady state of contraction without expenditure of energy.
was also found that during the course of normal tonic contraction the
ency of electrical activity decreases progressively with time whereas the
of relaxation after a period of tonus does not decrease. These observa-
point to the possibility that the 'viscosity' of this type of muscle may be
ased by motor and decreased by inhibitory nerve impulses. This hypo-
s is now being tested by Dr Lowy in experiments with preparations of
etractor muscles of *Mytilus*, the adductor muscles of *Mya arenaria* and
wo portions of the adductor muscle of *Pecten.* It is hoped to publish
nplete account of the results in the near future.

r D. B. Carlisle has continued work started in Oxford and Naples
erning the endocrinology of the pituitary of ascidians and that of the

eyestalks of crustaceans, especially with regard to the physiology of moulting and of sex.

Fish and Fisheries

A third paper—on age and growth rate—has been completed by Mr G. A. Steven in continuation of his work on mackerel biology. Owing to difficulty of age determination in this fish the age and growth had not previously been satisfactorily determined. Two schools of thought arose, one attributing a length of little over 10 cm. at the end of the first year of life while the other ascribed lengths of up to 25 cm. to 1-year-old fish. Mr Steven's results provide firm support for the second school. Direct age readings from scales and otoliths have been made of fish up to 6 years of age, and the probable sizes at greater ages have been calculated. It would seem that mackerel have a normal life span of 20 years or more.

The possibility of forecasting fishing prospects in the Cornish deep sea fishery is now being investigated, with promising initial results. Purely tentative assessments indicate that the prospects for good fishing in 1952 are in the order of 14 as against 1 for 1950 and 21 for 1949. No suitable data upon which to assess the 1951 prospects were obtained. Unfortunately this fishery is so greatly influenced by marketing conditions that actual yields may have little relation to natural stocks.

A beginning has also been made with a trawling survey of grounds in the vicinity of Plymouth for comparison with exactly comparable surveys made in 1913–14 and in the early 1920's. It is too early yet to draw firm conclusions from this work; but preliminary trends suggest present catches to be poorer than those of the 1920's but better than those of the 1913–14 period.

Mr P. G. Corbin has continued the collection of sand-eels with a trawl fitted with a fine mesh cover on the cod-end. Good samples of two off-shore species, *Ammodytes immaculatus* and *Gymnammodytes semisquamatus*, have now been accumulated and from this material it is hoped to be able to work out something of their life histories.

Mr P. G. Corbin, in collaboration with Mr G. A. Steven, has continued the development and trials of the new type of ringless conical net referred to in last year's Report of the Council. A full scale model 30 ft. (*c.* 9·1 m.) in diameter and 90 ft. (*c.* 27·5 m.) long, has now been delivered from the makers. Trials will be started as soon as the rigging of the net is finished.

In addition to an attempt to fill a gap in our knowledge of the life histories of food fishes by capturing the small fast-swimming stages, one of the original objectives has been to design a large pelagic net to fish in oceanic depths for some of the larger organisms known to be present, but which have hitherto escaped capture in any numbers. For this, a large net is required which is at the same time convenient to handle: it will also be necessary to fish the net at some speed. There will be a high frictional drag on a large conical net and

ng lifting effect which will increase rapidly with increasing towing
s. It seems improbable then that strictly horizontal hauls with a 30 ft.
ter conical net will be able to be made in deep water owing to the lift
net of this size, although vertical and oblique hauls will be possible.
er does the use of a depressor, or a series of depressors, seem to offer
ticable means of keeping down so large a net. A solution of the depth-
ng problem appears, however, to be within sight as a result of work by
orbin on the design and development of a self-depressing version of the
ss net. An 8 ft. diameter (*c.* 2·4 m.) model has been used in performance
and gave very efficient depression of a 1¾ in. circumference steel trawl
when fishing at speeds up to 6 knots. The stability of the net and
was not affected by towing in tight S-bends and turns. It is hoped to
further trials with a considerably larger model of this self-depressing net.

ther tests on preservatives for nets, trawl twines and ropes are being
d out by Dr Atkins and Mr F. J. Warren at Pier Cellars. Two of the
preservatives have given good results on duplicate series of nets. None
twines or ropes has failed as yet.

ry

thanks of the Association are again due to many foreign Government
rtments, to Universities and to other Institutions at home and abroad
pies of books and current numbers of periodicals either presented to
brary or received in exchange for the *Journal* of the Association.

nks are also due to those who have sent books or reprints of their
s, which are much appreciated.

M. V. Lebour has most generously presented to the Library a valuable
tion of reprints and books, including many works on parasitology,
cea, mollusca and plankton, which will prove of great use to workers
laboratory. Professor L. F. de Beaufort has been kind enough to
ge for the Library to receive all future Reports of the Siboga Expedition,
nplete the set bequeathed to us by the late Sir Sidney Harmer.

Library has again been much used by visiting members of the
ation.

shed Memoirs

ume xxx, No. 1, of the *Journal* was published in June 1951, No. 2 in
er 1951 and No. 3 in February 1952.

following papers, the outcome of work done at the laboratory, have
published elsewhere than in the *Journal* of the Association:

T, B. C. & Ritchie, J. M., 1951. The onset of shortening in striated muscle.
urn. Physiol.*, Vol. 113, pp. 336–45.

NDROWICZ, J. S., 1951. Muscle receptor organs in the abdomen of *Homarus vulgaris*
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ership of the Association

total number of members on 31 March 1952 was 659, being 47 more
n 31 March 1951; of these the number of life members was 84 and
ual members 575. The number of Associate members is six.

ce

eral Fund. The thanks of the Council are again due to the Development
missioners for their continued support of the general work of the
tory.

ital Grant. The Council wish to record their thanks to the Develop-
Commissioners for a capital grant to meet the cost of building the
laboratory for research with radioactive substances.

ate Income. The Council gratefully acknowledge the following generous
for the year:

m the Fishmongers' Company (£425), the Royal Society (£50), British
iation (£50), Physiological Society (£30), the Cornwall Sea Fisheries
ittee (£10), the Universities of London (£210), Cambridge (£125),
d (£100), Bristol (£50), Birmingham (£31. 10*s*.), Leeds (£20), Durham
10*s*.), Manchester (£10. 10*s*.), Sheffield (£10. 10*s*.), Nottingham
10*s*.), Exeter (£10. 10*s*.), Leicester (£10. 10*s*.), Hull (£10. 10*s*.),
ampton (£10. 10*s*.), and the Imperial College of Science and Technology
.

President, Vice-Presidents, Officers and Council

The following is the list of those proposed by the Council for election f the year 1952–53

President

Prof. James Gray, C.B.E., M.C., Sc.D., LL.D., F.R.S.

Vice-Presidents

The Earl of Iveagh, C.B., C.M.G.
Viscount Astor
Sir Nicholas E. Waterhouse, K.B.E.
Col. Sir Edward T. Peel, K.B.E., D.S.O., M.C.
G. P. Bidder, Sc.D.
W. T. Calman, C.B., D.Sc., F.R.S.
Vice-Admiral Sir John A. Edgell, K.B.E., C.B., F.R.S.
Prof. A. V. Hill, C.H., O.B.E., Sc.D., F.R.S.
E. S. Russell, O.B.E., D.Sc.
Sir Edward J. Salisbury, Kt., C.B. D.Sc., Sec.R.S.
Admiral Sir Aubrey C. H. Smit K.B.E., C.B., M.V.O.
A. T. A. Dobson, C.B., C.V. C.B.E.
Major E. G. Christie-Miller
Morley H. Neale, C.B.E.
The Rt. Hon. Major Sir Thomas Dugdale, Bt., M.P.

COUNCIL

To retire in 1953

G. E. R. Deacon, D.Sc., F.R.S.
E. Ford
F. C. Fraser, D.Sc.
Prof. J. E. Harris, Ph.D.
Prof. C. M. Yonge, D.Sc., F.R.S.

To retire in 1954

J. S. Colman
H. Cary Gilson
Prof. Alastair Graham, D.Sc.
N. A. Mackintosh, C.B.E., D.Sc.
Prof. J. Z. Young, F.R.S.

To retire in 1955

Prof. H. Graham Cannon, Sc.D., F.R.
O. D. Hunt
Prof. O. E. Lowenstein, Ph.D.
G. P. Wells, Sc.D.
R. S. Wimpenny

Hon. Treasurer

Major E. G. Christie-Miller, 38 Hyde Park Street, London, W. 2

Secretary

F. S. Russell, D.S.C., D.F.C., F.R.S., The Laboratory, Citadel Hill, Plymout

The following Governors are also members of the Council:

G. P. Bidder, Sc.D.
H. J. Johns, C.B., M.B.E. (Ministry of Agriculture and Fisheries)
The Worshipful Company of Fishmongers:
  The Prime Warden
  Major E. G. Christie-Miller
  Harrison S. Edwards
Prof. A. C. Hardy, D.Sc., F.R.S. (Oxfo University)
C. F. A. Pantin, Sc.D., F.R.S. (Car bridge University)
Edward Hindle, Sc.D., F.R.S. (Briti Association)
H. W. Parker, D.Sc. (Zoological Societ
Prof. A. V. Hill, C.H., O.B.E., Sc. F.R.S. (Royal Society)

BALANCE SHEET 1951–52

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31st March 1952

31st March 1951			£	£
	Capital Reserve Account:			
	As at 31st March 1951		32,688	
32,688	*Add:* Grants received and receivable from the Development Fund in respect of cost to date of new Research Vessel		6,216	38,904
	Surplus Account:			
	As at 31st March 1951		6,903	
6,903	*Add:* Excess of Income over Expenditure for the year		1,331	8,234
39,591				47,138
	Aquarium Sinking Fund:			
	As at 31st March 1951		224	
224	*Add:* Donations for rebuilding Aquarium Tanks ...		28	252
		£		
	E. T. Browne Bequest Funds:			
	Library Fund, as at 31st March 1951	1,189		
	Add: Interest on Investment	36	1,225	
	Special Apparatus Fund, as at 31st March 1951	2,586		
	Add: Interest on Investment	81		
		2,667		
	Less: Expenditure during the year	34	2,633	
	Scientific Publications Fund, as at 31st March 1951	2,169		
5,945	*Add:* Interest on Investment	61	2,230	6,088
—	Royal Society Grant—Publication of Monograph on Medusae of British Isles ...		750	
	Add: Bank Deposit Interest credited during year		3	753
	Composition Fees Fund:			
	As at 31st March 1951		857	
857	*Add:* Fees received during year		63	920
	Buildings Reconstruction Fund:			
	As at 31st March 1951		176	
	Add: War Damage Compensation receivable during year		792	
			968	
176	*Less:* Expenditure during year		160	808

31st March 1951			£	£
	Fixed Assets, at valuations as estimated by the Director at 31st March 1952:			
	Boats and Equipment:			
11,000	F.V. 'Sula'		11,000	
200	Motor Boat 'Gammarus'		200	
100	Nets, Gear and General Equipment		100	
11,300			11,300	
8,851	Laboratory Apparatus, Equipment and Machinery		9,315	
17,887	Library at valuation of Mr Ridgill Trout in January 1941, plus additions at cost		18,687	
38,038			39,302	
—	New Research Vessel (in course of construction). Cost to 31st March 1952		6,216	
38,038				45,518
232	General Fund Investment at Book Value £352. 2*s.* 3*d.* 2½% Treasury Stock ... ... (Market Value £204. Last year £238)			232
5,795	E. T. Browne Bequest Fund Investments, at cost £5901. 8*s.* 7*d.* British Transport 3% Guaranteed Stock 1978/88 ... ... (Market Value £4544. Last year £5223)			5,795
	Composition Fees Fund Investments, at cost:			
	£18. 8*s.* 6*d.* 2½% Treasury Stock		16	
857	£932. 15*s.* 9*d.* British Transport 3% Guaranteed Stock 1978/88 ... ... (Market Value £729. Last year £784)		888	904
	Current Assets:			
	Stocks on Hand as valued by the Director:			
	Specimens	600		
	Chemicals	250		
1,250	Journals	400	1,250	
	Sundry Debtors:			
	Sale of Specimens, Journals, Nets and Hydrographical Gear	1,015		
	Recoverable Expenditure	70		
1,785	War Damage Compensation receivable ...	140	1,225	
89	Prepayments		65	
	Balance at Bank and Cash in Hand:			
	Coutts & Co., London			
	Current Account	6,590		
	Deposit Account	753		
		7,343		
	Lloyds Bank Ltd., Plymouth	845		
(−183)	Cash in Hand	58	8,246	10,786

Notes:

1. The Association had a capital commitment at 31st March 1952 in respect of a new Research Vessel amounting to £112,000 which will be recoverable under a Development Fund Grant.

Plymouth
13th May 1952.

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH 1952

Year ended 31st March 1951		£	£
17,275	SALARIES, including Association's contributions to Superannuation Scheme and National Insurance		20,321
12,828	LABORATORY AND BOATS' CREWS' WAGES, including National Insurance, contributions to Superannuation Scheme, War Bonus and Employer's Liability Insurance		15,716
765	UPKEEP OF LIBRARY		677
1,706	SCIENTIFIC PUBLICATIONS, *less* sales		1,692
	UPKEEP OF LABORATORIES AND AQUARIUM:		
479	Buildings and Machinery	734	
651	Electricity, Gas, Coal and Water	914	
1,159	Chemicals and Apparatus	1,148	
136	Insurances, Tithe, Ground Rent and Rent of Store ...	159	
587	Travelling Expenses	572	
74	Audit Fee	63	
764	Stationery, Postages, Telephone, Carriage and Sundries	867	
207	Specimens	247	
			4,704
	MAINTENANCE AND HIRE OF BOATS:		
633	Petrol, Oil, Paraffin, etc.	741	
1,486	Maintenance and Repairs to Nets, Gear and Apparatus	1,762	
224	Boat Hire, Collecting Expenses and Upkeep of Truck	176	
690	Insurances	659	
1,380	Hire of R.V. 'Sabella'	1,380	
			4,718
40	ENTERTAINMENT EXPENSES		47
35	BANK CHARGES		31
2,223	BALANCE, being Excess of Income over Expenditure for the year		1,331
£43,342			£49,237

Note: No provision is made for replacement of Fixed Assets.

Year ended 31st March 1951			£	£
	GRANTS AND TABLE RENTS:			
36,350	Ministry of Agriculture and Fisheries grant from Development Fund		42,000	
500	Fishmongers' Company		450	
1,273	Miscellaneous (including British Association £50, Royal Society £50, Physiological Society £30, Cornwall Sea Fisheries Committee £10, Universities of: London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s.* 0*d.*, Leeds £20, Durham £10. 10*s.* 0*d.*, Exeter £10. 10*s.* 0*d.*, Leicester £10. 10*s.* 0*d.*, Manchester £10. 10*s.* 0*d.*, Nottingham £10. 10*s.* 0*d.*, Southampton £10. 10*s.* 0*d.*, Hull £10. 10*s.* 0*d.*, Sheffield £10. 10*s.* 0*d.*, Imperial College £10, and Ministry of Works £104) ...		1,125	
				43,575
514	SUBSCRIPTIONS (excluding subscriptions received in advance)			540
65	FEES FOR TESTS OF MATERIALS			46
	SALES:			
2,350	Specimens		2,633	
410	Fish		680	
	Nets, Gear and Hydrographical Apparatus ...	1,168		
	Less: Cost of Materials	730		
428			438	
				3,751
31	INTEREST ON INVESTMENTS			36
6	SALE OF DR M. V. LEBOUR'S BOOK			5
7	SALE OF 'PLYMOUTH MARINE FAUNA'			2
	AQUARIUM:			
	Admission Fees		1,575	
	Sales of Guides and Postcards		114	
			1,689	
	Less: Maintenance of Building	17		
	Printing Guides and Tickets	130		
	Advertising	58		
	Food	107		
	Wages	95		
			407	
1,408				1,282
£43,342				£49,237

T OF GOVERNORS, FOUNDERS, MEMBERS, IONORARY AND ASSOCIATE MEMBERS

1952

GOVERNORS

itish Association for the Advancement of Science, *Burlington House, W.* 1
niversity of Oxford
niversity of Cambridge
orshipful Company of Clothworkers, 48 *Fenchurch Street, E.C.* 3
orshipful Company of Fishmongers, *London Bridge, E.C.* 4
Prime Warden. (**Council**, 1886→)
ards, Harrison S., *Westhumble Lacey, nr Dorking, Surrey*. (**Council**, 1950→)
stie-Miller, Major E. G., 38 *Hyde Park Street, W.* 2. (**Council**, 1941→;
on. Treasurer, 1941→; **Vice-President**, 1951→)
ological Society of London, *Regent's Park, N.W.* 8
yal Society, *Burlington House, Piccadilly, W.* 1
ry of Agriculture and Fisheries, 3 *Whitehall Place, S.W.* 1
Robert (the late). (**Council**, 1896–1901)
John (the late)
e, E. T. (the late). (**Council**, 1913–19; 1920–37)
sson, J. P. (the late). (**Council**, 1896–1903)
, G. P., Sc.D., *Cavendish Corner*, 221 *Hills Road, Cambridge*. (**Council**,
99→; **President**, 1939–45; **Vice-President**, 1948→)
ord Moyne, P.C., D.S.O. (the late). (**Vice-President**, 1929; 1939–45;
esident, 1930–39)
E. J., C.B.E., D.Sc., LL.D., F.R.S. (the late) (Honorary.) (**Council**,
95–1942; **Secretary**, 1895–1936; **Hon. Governor**, 1937–42)

FOUNDERS

he Corporation of the City of London, *The Guildhall, E.C.* 3
he Worshipful Company of Mercers, *Mercers' Hall*, 4 *Ironmonger Lane, E.C.* 2
he Worshipful Company of Goldsmiths, *Goldsmiths' Hall, Foster Lane, E.C.* 2
he Royal Microscopical Society, *B.M.A. House, Tavistock Square, W.C.* 1
ulteel, Thos. (the late)
urdett-Coutts, W. L. A. Bartlett (the late)
risp, Sir Frank, Bart. (the late). (**Council**, 1884–92; **Hon. Treasurer**, 1884–88)
aubeny, Captain Giles A. (the late)
ddy, J. Ray (the late)
assiott, John P. (the late)
ankester, Sir E. Ray, K.C.B., F.R.S. (the late). (**Hon. Secretary**, 1884–90;
President, 1891–1929)
ord Masham (the late)

1884 Moseley, Prof. H. N., F.R.S. (the late). (**Chairman of Council, 1884–**
1884 Lord Avebury, F.R.S. (the late). (**Vice-President**, 1884–1913)
1884 Poulton, Prof. Sir Edward B., F.R.S. (the late). (**Council**, 1888–94)
1884 Romanes, Prof. G. J., LL.D., F.R.S. (the late). (**Council**, 1884–91)
1884 Worthington, James (the late)
1885 The 15th Earl of Derby (the late)
1887 Weldon, Prof. W. F. R., F.R.S. (the late). (**Council**, 1890–1901; represent
British Association, 1901–5)
1888 Bury, Henry, *The Gate House*, 17 *Alumdale Road, Bournemouth West*
1888 The Worshipful Company of Drapers, *Draper's Hall, E.C.* 2
1889 The Worshipful Company of Grocers, *Grocers' Hall, Princes Street, E.C*
1889 Thompson, Sir Henry, Bart. (the late). (**Vice-President**, 1890–1903)
1889 Lord Revelstoke (the late)
1890 Riches, T. H. (the late). (**Council**, 1920–25)
1892 Browne, Mrs E. T. (the late)
1898 Worth, R. H., M.Inst.C.E., (the late)
1899 The Earl of Iveagh, C.B., C.M.G., 11 *St James's Square, S.W.* 1. (**Vi**
President, 1929→)
1902 Gurney, Robert, D.Sc. (the late). (**Council**, 1932–5)
1904 Shaw, Joseph, K.C. (the late)
1909 Harding, Colonel W. (the late)
1910 Murray, Sir John, K.C.B., F.R.S. (the late). (**Council**, 1896–99; **Vi**
President, 1900–13)
1912 Swithinbank, H. (the late)
1913 Shearer, Dr Cresswell, F.R.S. (the late)
1913 Heron-Allen, E., F.R.S. (the late)
1918 Evans, George (the late). (**Hon. Treasurer**, 1915–31; **Vice-Preside**
1925–33)
1920 McClean, Capt. W. N., 39 *Phillimore Gardens, W.* 8
1920 Lord Buckland of Bwlch (the late)
1920 Llewellyn, Sir D. R. (the late)
1921 Harmer, F. W. (the late)
1924 The MacFisheries, Ltd., *Ocean House, Pudding Lane, E.C.* 3
1924 Lady Murray (the late)
1925 The Institution of Civil Engineers, *Great George Street, Westminster, S.W*
1925 Discovery Committee
1927 Bidder, Miss Anna M., Ph.D., *Cavendish Corner*, 221 *Hills Road, Cambri*
(**Council**, 1948–51)
1933 Peel, Col. Sir Edward T., K.B.E., D.S.O., M.C., *c/o Messrs Peel and Co.,*
P.O. Box 331, *Alexandria, Egypt*. (**Vice-President**, 1936→)
1938 Buchanan, Dr Florence (the late)
1945 Brown, Arthur W. W. (the late)

MEMBERS

* Life Members

1949 Abbott, B. C., *Biophysics Department, University College, Gower Str*
London, W.C. 1
1945 Aberdeen University Library, *The University, Aberdeen*
1934 Adam, Mrs K. M. G., 84 *Lasswade Road, Edinburgh* 9
1951 Adams, E., 2 *Woodford Crescent, Marsh Mills, Plympton, Devon*

Adrian, Prof. E. D., O.M., M.D., D.Sc., LL.D., P.R.S., *St Chad's, 48 Grange Road, Cambridge*
Affleck, R. J., *Snob's Creek Fish Hatchery, Private Mail Bag, via Alexandra, Victoria, Australia*
Aleem, A. A., Ph.D., *Faculty of Science, Farouk I University, Moharram Bey, Alexandria, Egypt*
Alexandrowicz, J. S., Ph.D., M.D., *The Laboratory, Citadel Hill, Plymouth, Devon*
Allen, J. A., *Department of Zoology, The University, Glasgow, W. 2*
Allen, Miss J. M., *Tenements Farm, Chipperfield, Herts.*
Allen, Mrs M. E., 82 *Oaks Avenue, Worcester Park, Surrey*
Amirthalingam, C., Ph.D., 2 *Dickmans Path, Colombo, Ceylon*
Arnold, D. C., *Fair Acre, Abbotskerswell, Newton Abbot, Devon*
Ashby, D. G., 195 *Chesterton Road, Cambridge*
Viscount Astor, 3 *Elliot Terrace, Plymouth, Devon.* (**Vice-President, 1911→**)
Atkins, Miss D., D.Sc., *The Laboratory, Citadel Hill, Plymouth, Devon*
Atkins, W. R. G., C.B.E., Sc.D., F.R.I.C., F.Inst.P., F.R.S., *The Old Vicarage, Antony, Torpoint, Cornwall*
Atkinson, G. T., *Gresham House, Esplanade, Lowestoft, Suffolk*
Atlantic Biological Station, *St Andrews, N.B., Canada*
Attridge, J., 44 *Windermere Road, Muswell Hill, London, N. 10*

Baal, H. J., 3 *Bel Royal Villas, Jersey, C.I.*
Baerends, Prof. G. P., *Zoological Laboratory, Reitemakersrijge 14, Groningen, Holland*
Bagenal, T., *Marine Station, Keppel Pier, Millport, Isle of Cumbrae*
Bahl, Prof. K. N., D.Sc., *Department of Zoology, The University, Lucknow, India*
Baily, Joshua L. Jr., 4435 *Ampudia Street, San Diego 3, Calif., U.S.A.*
Bainbridge, R., 43 *Strathmore Avenue, Hull*
Baker, J. R., D.Sc., *Department of Zoology and Comparative Anatomy, University Museum, Oxford*
Baldwin, Prof. E., Ph.D. *Department of Biochemistry, University College, Gower Street, London, W.C. 1.* (**Council, 1946–48**)
Ballantine, Miss D., *Priors Close, Cambo Place, Cullercoats, Northumberland*
Bangor, Marine Biological Station, *University College of North Wales, Bangor, Caern*
Barnard, E. E. P., 7 *Webster Gardens, Ealing, London, W. 5*
Barnes, H., Ph.D., *Marine Station, Keppel Pier, Millport, Isle of Cumbrae*
Barrington, Prof. E. J. W., D.Sc., *Department of Zoology, The University, Nottingham*
Barron, H., 65 *Sumerton Road, Belfast, N. Ireland*
Barter, W. Y., 29 *Sea View Avenue, Plymouth, Devon*
Bassindale, R., *Department of Zoology, The University, Bristol*
Batham, Miss E. J., Ph.D., *Portobello Marine Biological Station, via Port Chalmers, Otago, New Zealand*
Baughman, J. L., *Texas Game, Fish and Oyster Commission, Rockport, Texas, U.S.A.*
Baxter, E. W., *Biology Department, Medical School, Guy's Hospital, London, S.E. 1*
Baylis, L. E., Ph.D., *Department of Physiology, University College, Gower Street, London, W.C. 1*

1934 Beadle, L. C., *Department of Biology, University College of East Afr* *P.O. Box 262, Kampala, Uganda*
1928 Beer, G. R. de, D.Sc., F.R.S., *British Museum (Natural History), Crom* *Road, London, S.W. 7*
1950 Bell, Mrs E. B., *Solva, Glanford Road, Brigg, Lincs*
1947 Berrill, Prof. N. J., F.R.S. *Department of Zoology, McGill Univers* *Montreal, Canada*
1947 Best, A. C. G., 6 *Station Road, Loudwater, High Wycombe, Bucks*
1948 Betts, Slade, 100 *Avondale Road, Bromley, Kent*
1903 Bidder, Col. H. F., *The Malting House, Nettlebed, near Henley-on-Tham* *Oxon*
*1945 Bingley, F. J., *Flatford Mill Field Centre, East Bergholt, near Colchester, Es*
1925 Birkbeck College, *Malet Street, London, W.C.* 1
1951 Birkett, L., *Fisheries Laboratory, Lowestoft, Suffolk*
1931 Birtwistle, W., 73 *North Street, Skibbereen, Co. Cork, Eire*
1947 Bishop, M. W. H., *Meadow Farm, Waterbeach, Cambs*
1945 Black, J. A., *Ash House, Caton, near Lancaster, Lancashire*
1947 Black, Miss M. K., *c/o F. Band, High Street, Benwick, March, Cambs*
1951 Blackburn, M., *C.S.I.R.O. Fisheries Division, Cronulla, N.S.W., Austral*
1930 Blaschko, Dr H., *Department of Pharmacology, South Parks Road, Oxfo*
1952 Blaxter, J. H. S., *Pathside, Frithesden Copse, Berkhamsted, Herts*
1910 Bloomer, H. H., *Longdown, Sunnydale Road, Swanage, Dorset*
1951 Boden, B. P., Ph.D., *Bermuda Biological Station, St George's West, Berm*
1936 Bogue, Prof. J. Yule, D.Sc., *Heyscroft, Hartley Road, Altrincham, Chesh*
1932 Bolitho, Capt. R. J. B., *Gorey, Jersey, C.I.*
1945 Boney, A. D., 1 *Whiteford Road, Mannamead, Plymouth, Devon*
*1933 Boschma, Prof. Dr H., *Rijksmuseum van Natuurlijke Historie, Leiden, Holl*
1947 Bossanyi, J., *Dove Marine Laboratory, Cullercoats, Northumberland*
1949 Braithwaite, E. R., *Research Department, Acheson Colloids Ltd., Prince R* *Plymouth, Devon*
1940 Brambell, Prof. F. W. Rogers, D.Sc., F.R.S., *Department of Zoology, Univer* *College of North Wales, Bangor, Caern.* (**Council**, 1944–47, 1948–51)
1924 Brightwell, L. R., 1 *Edith Avenue, Peacehaven, Sussex*
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1941 British Celanese Ltd., *Celanese House, Hanover Square, London, W.* 1
1948 British Cod Liver Oils (Hull and Grimsby) Ltd., *P.O. Box No.* 18, *Hul*
1939 British Ropes Ltd., *Western Avenue, Cardiff*
*1946 Brock, Mrs C. H., Ph.D., *Dept. of Agriculture, Downing Street, Cambri*
1946 Brough, Prof. James, D.Sc., *Department of Zoology and Comparative Anato* *University College, Newport Road, Cardiff*
1928 Brown, Miss E. M., Ph.D., 6 *Effingham Lodge, Surbiton Crescent, Kingst* *on-Thames, Surrey*
1936 Brown, Herbert H., O.B.E., Ph.D., *Fisheries Division, F.A.O. of U.N., V* *delle Terme di Caracalla, Rome, Italy*
*1925 Bull, Herbert O., D.Sc., *Dove Marine Laboratory, Cullercoats, No* *umberland*
1951 Burgess, G. H. O., *Department of Zoology, University College, Hull*
1920 Burne, R. H., F.R.S., *c/o Westminster Bank*, 329 *High Holborn, Lon* *W.C.* 1
1948 Burrows, Mrs E. M., Ph.D., *Hartley Botanical Laboratories, The Univers* *Liverpool* 3
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Carter, G. S., Ph.D., *Department of Zoology, Downing Street, Cambridge*
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Clair, C. le, *Glebe Cottage, Cornwood, Ivybridge, Devon*
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1939 Clowes, A. J., *Division of Fisheries, Beach Road, Sea Point, Cape Tow S. Africa*
*1886 Coates and Co. (Plymouth), Ltd., *Black Friars Distillery, Southside Stre Plymouth, Devon*
*1945 Cobham, Lt.-Cdr. A. J., R.N., *Noel Cottage, Castle Street, Portchester, Ha*
*1925 Cockshott, Lt.-Col. A. M., R.A.S.C., *Cotteswold Naturalists' Field Cl City Library, Gloucester*
1933 Cole, H. A., D.Sc., *Fisheries Experiment Station, Castle Bank, Conwa Caern*
*1948 Collier, Albert, *c/o Fish and Wildlife Service, Ft Crockett, Galveston, Texa U.S.A.*
*1885 Collier and Co., 53 *Southside Street, Plymouth, Devon*
1950 Collins, William N., 603 *Thatcher Avenue, River Forest, Illinois, U.S.A.*
1947 Collis, Miss M. M., 27 *Mowbray Road, Cambridge*
1930 Colman, J. S., *Marine Biological Station, Port Erin, Isle of Man.* (**Counci** 1951→)
1947 Cook, Miss P. M., 51 *Runnymeade Crescent, Streatham, London, S.W.* 16
1940 Cook, R. H., *Moor Close, Melbourn, Cambridge*
*1933 Cooper, L. H. N., D.Sc., F.R.I.C., *The Laboratory, Citadel Hill, Plymout Devon*
1937 Corbin, P. G., *The Laboratory, Citadel Hill, Plymouth, Devon*
1937 Corbin, Mrs P. G., Ph.D., *Dostabrook, Horrabridge, Devon*
1946 Corlett, John, M.Sc., *Fisheries Laboratory, Lowestoft, Suffolk*
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1952 Cowper, T. R., *C.S.I.R.O. Fisheries Division, Cronulla, N.S.W., Australi*
1936 Crawford, G. I., 18 *East Drive, Carshalton Beeches, Surrey*
1952 Crawshaw, K. Ridgway, *Heron Cottage, White Notley, Witham, Essex*
*1928 Crew, Prof. F. A. E., M.D., D.Sc., F.R.S., *Usher Institute, Warrenden Pa Road, Edinburgh* 9
*1952 Crisp, D. J., Ph.D., *Bryn Hydd, Holyhead Road, Bangor, Caern*
1929 Crofts, Miss D. R., D.Sc., *Deerbank, Noisey Wood, Billericay, Essex*
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1948 Dales, R. Phillips, Ph.D., 67 *Westmoreland Avenue, Squirrels Heath, Esse*
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*1919 Damant Capt. G. C. C., C.B.E., R.N., *Thursford, Cambridge Road, E Cowes, I. of W.* (**Council**, 1928–31, 1937–40)
1939 Danielli, Prof. J. F., D.Sc., *Department of Zoology, King's College, Stra London, W.C.* 2. (**Council**, 1944–45)
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Dugdale, Rt. Hon. Major Sir Thomas L., Bt., M.P., *Ministry of Agriculture and Fisheries*, 55 *Whitehall, London, S.W.* 1. (**Vice-President**, 1952→)
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Egypt: Coastguard and Fisheries Service, *Alexandria, Egypt*
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Faulkner, I. J., Ph.D., *I.C.I. Billingham Division, Billingham, E. Durham*

1950 Featherstone, Miss M. J., 3 *Clarence Road, Bickley, Kent*
1950 Felinska, Mrs Maria, Dr Phil., *Stowell Park, near Cheltenham, Glos*
*1933 Fellowes, Miss Rosalind, 23 *The Cloisters, Windsor Castle, Berks*
1950 Fisher, L. R., 23 *Langton Avenue, Chelmsford, Essex*
1940 Foote, Miss V. V. J., *Newent Court, Newent, Glos*
1928 Ford, E., *Marine Station, Keppel Pier, Millport, Isle of Cumbrae.* (**Counci** 1950→)
1935 Ford, E. B., D.Sc., F.R.S., *Department of Zoology and Comparative Anatom University Museum, Oxford*
1952 Ford, Miss V. E., 60 *Dudley Road, Hollingbury, Brighton, Sussex*
1939 Forrest, J. E., *Department of Zoology, Queen Mary College, Mile End Roa London, E.* 1
1950 Forster, G. R., *The Laboratory, Citadel Hill, Plymouth, Devon*
1949 Fountain, H. C., *The Store Cottage, Polbathic, Torpoint, Cornwall*
1912 Fox, Prof. H. M., F.R.S., *Bedford College for Women, Sussex Lodge, Regent Park, London, N.W.* 1. (**Council**, 1928–30, 1931–34, 1944–47)
1942 Foxon, G. E. H., *Department of Biology, Guy's Hospital Medical Schoo London Bridge, London, S.E.* 1
1950 Frampton, Cdr R. H. C. F., R.N. (Rtd), *Ministry of Agriculture and Fisherie Citadel Hill, Plymouth, Devon*
1924 Fraser, Miss E. A., D.Sc., *Orchard Cottage, Abinger Common, Dorking, Surre*
1935 Fraser, F. C., D.Sc., *British Museum (Natural History), Cromwell Roa London, S.W.* 7. (**Council**, 1950→)
*1935 Fraser, James H., Ph.D., *Marine Laboratory, Wood Street, Torry, Aberdee*
*1939 Fretter, Miss Vera, D.Sc., *Department of Zoology, Birkbeck College, Mal Street, London, W.C.* 1
*1930 Fritsch, Prof. F. E., D.Sc., F.R.S., 34 *Causewayside, Cambridge.* (**Counci** 1931–34, 1937–40, 1943–46)
1949 Fuller, A. S., 30 *Staines Avenue, Cheam, Surrey*
1948 Furness, W. J., *Inglewood, Abbey Park Road, Grimsby, Lincs*

1941 Gardiner, Mrs A. C., 6 *Klementinenstrasse, Munich*, 23, *Germany*
1949 Gates, J. T., 19 *Fircroft Road, Hook Rise, Surbiton, Surrey*
*1928 Gates, Prof. R. R., D.Sc., LL.D., F.R.S., *Biological Laboratories, Harvar University, Cambridge* 38, *Mass., U.S.A.*
1948 Gatty Marine Laboratory, (The Principal), *The University, St Andrews, Fi*
1950 Gauld, D. T., Ph.D., *Marine Station, Millport, Isle of Cumbrae*
1935 Gilson, H. Cary, *Freshwater Biological Association, The Ferry Hous Far Sawrey, Ambleside, Westmorland.* (**Council**, 1940–43, 1947–5 1951→)
1951 Glaister, Mrs K., 12 *Grey Close, London, N.W.* 11
1945 Glasgow University, Zoology Department, *Glasgow, W.* 2
1951 Glasson, D. R., Ph.D., 34 *Craven Avenue, St Judes, Plymouth, Devon*
1946 Glover, R. S., *Oceanographical Laboratory*, 78 *Craighall Road, Edinburgh,*
1951 Gokhale, S. V., 1 *Church Road, Port Erin, Isle of Man*
1951 Gold Coast, University College of, *P.O. Box* 4, *Achimota, Gold Coast*
1951 Goodbody, I. M., *Natural History Department, Marischal College, Aberdee*
1950 Goodley, E. F. W., 2 *Thickthorn Lane, Laleham, Staines, Middlesex*
1939 Goodrich, Dr Helen Pixell, 12 *Park Town, Oxford*
1939 Gordon, Miss Isabella, D.Sc., *British Museum (Natural History), Cromwe Road, London, S.W.* 7
1943 Gourock Ropework Co., Ltd., 92 *Bay Street, Port Glasgow, Renfrew*

Graham, Prof. Alastair, D.Sc., *Department of Zoology, Birkbeck College, Malet Street, London, W.C.* 1. (**Council**, 1951→)
Graham, F. G., *Trethake, Plymstock Road, Oreston, near Plymouth, Devon*
Graham, Michael, O.B.E., *Fisheries Laboratory, Lowestoft, Suffolk.* (**Council**, 1931–32, 1933–36, 1943–46, 1949–52)
Gray, Sir Archibald M. H., C.B.E., M.D., F.R.C.P., F.R.C.S., 39 *Devonshire Place, London, W.* 1
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Haswell, G. A., 23 *Russell Avenue, Hartley, Plymouth, Devon*
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1939 Hayes, Mrs F. R., *Dalhousie University, Halifax, N.S., Canada*
1950 Hazevoet, A. C., *Vogelenzangstraat* 19-*I*, *Amsterdam West, Holland*
1949 Heaysman, Miss J. E. M., 69 *Brook Road, Merstham, Surrey*
1948 Hedley, Ronald H., *Remora, Quarry House Lane, Crossgate Moor, Durham City*
1931 Henderson, G. T. D., D.S.C., Ph.D., *Oceanographic Laboratory*, 78 *Craighall Road, Edinburgh* 5
1925 Hentschel, C. C., 7 *Dudley Court, Upper Berkeley Street, London, W.* 1
1939 Herklots, G. A. C., Ph.D., *Vanners, Chobham, Surrey*
1950 Herring Industry Board, 1 *Glenfinlas Street, Edinburgh* 3
1939 Hewer, H. R., Assistant Professor, *Department of Zoology, Imperial College of Science, London, S.W.* 7
1926 Hickling, C. F., C.M.G., Sc.D., *Colonial Office, Sanctuary Buildings, Great Smith Street, London, S.W.* 1. (**Council**, 1947–50)
1951 Hidaka, Prof. K., *Geophysical Institute, Tokyo University, Tokyo, Japan*
1926 Hill, Prof. A. V., C.H., O.B.E., Sc.D., F.R.S., 16 *Bishopswood Road, Highgate, London, N.* 6. (**Council**, 1925–29, 1930–33, 1934–37, 1938–41, 1942→; representing Royal Society, 1944→; **Vice-President**, 1948→)
*1949 Hill, D. K., *Postgraduate Medical School of London, Ducane Road, London, W.* 12
1939 Hill, M. D., *Uplands, near Ledbury, Herefordshire*
1947 Hill, M. N., Ph.D., 6 *St Eligius Street, Cambridge*
*1921 Hindle, E., Sc.D, F.R.S., *Zoological Society of London, Regent's Park, London, N.W.* 8 (**Council**, 1946–49; representing British Association, 1950→)
1937 Hinton, M. A. C., F.R.S., *Glaister, Wrington, near Bristol, Somerset*
1926 Hobson, Prof. A. D., *King's College, Newcastle-upon-Tyne* 1, *Northumberland*
1948 Hockley, A. R., *Department of Zoology, The University, Southampton*
1939 Hodgkin, A. L., F.R.S., *Trinity College, Cambridge.* (**Council**, 1951–52)
1945 Hodson, W., *Rhodena, Penare Avenue, Prestatyn, Flints*
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1939 Holmes, W., D.Phil., *Department of Zoology and Comparative Anatomy, University Museum, Oxford*
1948 Holsgrove, H. E., 67 *Bridwell Road, Weston Mill Estate, Plymouth, Devon*
1950 Honeker, C., 24 *Romney Road, New Malden, Surrey*
1951 Hornby, Miss C. E., 53 *Langley Drive, Wanstead, London, E.* 11
1933 Horne, F. R., *National Institute of Agricultural Botany, Huntingdon Road, Cambridge*
1950 Horridge, G. A., *St John's College, Cambridge*
1952 Horvath, C., 8649 *Clifton Way, Beverley Hills, Calif., U.S.A.*
1950 Howard, G. H., 14 *Westlands Avenue, Bargate, Grimsby, Lincs*
1948 Howe, Surg. Cdr. (D) D. C., R.N., *The Cottage, Pomphlett, Plymouth, Devon*
1950 Howells, D. V., 42 *Radstock Road, Reading, Berks*
1932 Howes, N. H., *Department of Zoology, University College, Gower Street, London, W.C.* 1
1952 Hoyle, G., *Department of Zoology, The University, Glasgow, W.* 2
1928 Hunt, O. D., *Corrofell, Newton Ferrers, Devon.* (**Council**, 1944–47, 1948–51, 1952→)
1947 Hunter, W. Russell, *Department of Zoology, The University, Glasgow, W.* 2
*1947 Hurrell, H. G., J.P., *Moorgate, Wrangaton, Devon*
1939 Hurst, C. P., *Landulph Rectory, Saltash, Cornwall*
*1920 Hutton, J. Arthur, *Woodlands, Alderley Edge, Manchester*
*1952 Hutton, Robert F., *The Laboratory, Citadel Hill, Plymouth, Devon*

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Jones, Prof. R. V., C.B., C.B.E., D.Phil., F.Inst.P., *Department of Natural Philosophy, Marischal College, Aberdeen*
Jones, W. C., *King's College, Cambridge*
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Kay, Miss E. A., *Box* 522, *Kolva, Kanai, Territory of Hawaii, U.S.A.*
Keilin, Prof. D., Sc.D., F.R.S., *Molteno Institute, Cambridge.* (**Council**, 1940–43)
Keir, Ronald S., *Newfoundland Fisheries Research Station, Water Street East, St Johns, Newfoundland*

1946 Kelley, D. F., 17 *Bainbridge Avenue, Hartley, Plymouth, Devon*
1949 Kennedy, G. Y., Ph.D., F.R.I.C., *Department of Cancer Research, University Field Laboratory, Blackbrook Road, Sheffield* 10
1946 Kenya: The Game Warden, *Game Department, P.O. Box* 241, *Nairobi*
1951 Kerslake, Miss J., 17 *Hardman Road, Kingston-upon-Thames, Surrey*
1950 Keynes, R. D., Ph.D., *Physiological Laboratory, Cambridge*
1949 Kimmins, B. J., 19 *Cranleigh Gardens, Bristol* 9
1928 King, Mrs A. Redman, *Mixton House, Lerryn, Lostwithiel, Cornwall*
1947 Kingsbridge Modern Secondary School, *Kingsbridge, Devon*
1949 Kingston Technical College, *Kingston Hall Road, Kingston-upon-Thames, Surrey*
1927 Kirtisinghe, P., *Department of Zoology, University of Ceylon, Colombo* 3, *Ceylon*
1950 Kisch, B. S., C.I.E., 38 *Boulevard Thiers, St Jean-de-Luz, B.P., France*
1930 Kitching J. A., O.B.E., Ph.D., *Department of Zoology, The University, Bristol*
1939 Knight, Miss Margery, D.Sc., *University Hall for Women Students, Holly Road, Fairfield, Liverpool.* (**Council**, 1943–46)
1945 Knowles, F. G. W., D.Phil., *Marlborough College, Marlborough, Wilts*
1950 Korn, M. E., 62 *Aberdare Gardens, Hampstead, London, N.W.* 6
1950 Kott, Miss P., *C.S.I.R.O. Fisheries Division, Cronulla, N.S.W., Australia*
1948 Kow, Tham Ah, *c/o Fisheries Department, 4th Floor, Fullerton Building, Singapore*
1949 Kristensen, Dr Ingvar, *Zoölogisch Station, den Helder, Holland*

1950 La Rochelle: Station Océanographique, *Pavillon du Port-Le Gabut, La Rochelle (Charente-Maritime), France*
1950 Lasker, Reuben, *Hopkins Marine Station, Pacific Grove, Calif., U.S.A.*
*1925 Lebour, Miss M. V., D.Sc., *Kean Hill, Cawsand, near Plymouth, Devon*
1947 Leehane, J. D. B., 14 *Wyndham Street East, Plymouth, Devon*
1935 Le Mare, D. W., *Fisheries Department, Federation of Malaya and Singapore, Penang, Malaya*
1948 Letts, J. K., 183 *Windmill Lane, Greenford, Middlesex*
1951 Lewis, J. B., *Marine Laboratory, University of Miami, Coral Gables, Florida, U.S.A.*
1952 Lewis, J. R., *Department of Zoology, University College of Wales, Aberystwyth, Cardigan*
1951 Lightowlers, F., 109 *Bradford Road, Brighouse, Yorkshire*
1932 Lisbon: Instituto de Biologia Maritima, *Cais do Sodré, Lisbon, Portugal*
1952 Lister-Bradney, J., Ph.D., 25 *Palmerston Road, Westcliff-on-Sea, Essex*
1949 Lodge, Miss S. M., Ph.D., *Marine Biological Station, Port Erin, Isle of Man*
1950 Longhurst, A. R., *Landford Cottage, Landford, Wilts*
1948 Lovegrove, T., *Marine Laboratory, Wood Street, Torry, Aberdeen*
1950 Lowenstein, Prof. O. E., Ph.D., *Department of Zoology, The University, Birmingham*, 15. (**Council**, 1952→)
1926 Lowndes, A. G., Sc.D., F.R.I.C., *c/o The Laboratory, Citadel Hill, Plymouth, Devon*
1950 Lowy, J., Ph.D., *The Laboratory, Citadel Hill, Plymouth, Devon*
1931 Lucas, C. E., D.Sc., *Marine Laboratory, Wood Street, Torry, Aberdeen.* (**Council**, 1949–52)
1930 Lumley, Adrian, *Beechcroft, Udney Park Road, Teddington, Middlesex*
1938 Lysaght, Miss A. M., Ph.D., 6 *Cumberland Gardens, London, W.C.* 1

MacDonald, R., 112 *Antrim Road, Belfast, N. Ireland*
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Mackinnon, Prof. D. L., D.Sc., 44 *St Leonard's Terrace, Chelsea, London, S.W.* 3. (**Council**, 1938–42)
Mackintosh, N. A., C.B.E., D.Sc., 7 *Hinde House, Hinde Street, London, W* 1. (**Council**, 1946–49, 1951→)
Macnae, William, *Department of Zoology, Rhodes University College, Grahamstown, S. Africa*
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Matthews, L. Harrison, Sc.D., *Zoological Society of London, Regent's Park, London, N.W.* 8. (**Council**, 1944–47, 1949–52)
Mayne, Cyril F., O.B.E., F.R.C.S., *c/o Barclays Bank Ltd., Plymouth, Devon*
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Mexico: Department of Physiology, National University, *Apartado Postal* 13699, *Mexico, D.F.*
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Michelmore, Miss M. S. J., *Rowhorne House, Whitestone, Exeter, Devon*
Mij tot Exploitatie van Kooksovengassen (Mekog), *Ijmuiden, Holland*
Milford Haven Trawler Owners Association, Ltd., *Milford Haven, Pembs*
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1947 Mitra, G. N., *Assistant Director of Fisheries, Cuttack (Orissa), India*
1952 Mohamed, Prof. Abdel Fattah, Ph.D., *Oceanography Department, Faculty of Science, Farouk I University, Maharram Bey, Alexandria, Egypt*
1951 Mohr, J. L., Ph.D., *Department of Zoology, University of Southern California, Los Angeles 7, Calif., U.S.A.*
1940 Moore, Hilary B., Ph.D., *Marine Laboratory, Miami University, Coral Gables, Florida, U.S.A.*
1949 Morton, J. E., *Department of Zoology, Queen Mary College, Mile End Road, London, E.* 1
1931 Mount Desert Island Biological Laboratory, *Salisbury Cove, Maine, U.S.A*
1938 Mowbray, Louis L., *Curator, Bermuda Government Aquarium, Flatts, Bermuda*
1942 Moynahan, Dr E. J., *Wayside, Jordans, near Beaconsfield, Bucks*
1952 Munn, R. B., 11002 *East Saragossa Street, Whittier, Calif., U.S.A.*

1952 Naylor, E., *Marine Biological Station, Port Erin, Isle of Man*
1933 Neale, Morley H., C.B.E., *Chaffcombe House, Chard, Somerset.* (**Council**, 1934-36, 1939–42, 1943–46, 1947–50; **Vice-President**, 1951→)
1934 Neale and West, *Trawler Owners, Wharf Street, Cardiff*
1939 Needham, Joseph, Sc.D., F.R.S., *Gonville and Caius College, Cambridge*
1947 Newell, G. E., Ph.D., *Department of Zoology, Queen Mary College, Mile End Road, London, E* 1
1951 Newfoundland Fisheries Research Station, *Water Street East, St John's, Newfoundland*
1947 Newton, Prof. Lily, Ph.D., *Department of Botany, University College of Wales, Aberystwyth, Cardigan.* (**Council**, 1947–50)
1930 Nicholls, A. G., Ph.D., *C.S.I.R.O. Division of Fisheries, Stowell, Hobart, Tasmania*
1950 Nicol, J. A. C., D.Phil., *Trevean, George Lane, Plympton St Maurice, Devon*
1947 Nisbet, R. H., 8 *Lichfield Road, Woodford Green, Essex*
1948 Nobbs, Miss G. E. D., 4 *Clifton Estate, Plympton, Devon*
1949 Norris, E., *Biology Department, Kingston Technical College, Kingston Hall Road, Kingston-upon-Thames, Surrey*
1951 North Carolina, University of, *Fisheries Research Laboratory, Morehead City, N. Carolina, U.S.A.*
1950 North, J., 316 *Lee High Road, Lewisham, London, S.E.* 13
1952 Nunn, G., F.R.I.C., *Cleeve House,* 10 *Tyrells Close, Chelmsford, Essex*
1948 Nunn, S. M., *The Laboratory, Citadel Hill, Plymouth, Devon*
1950 Nurse, F. R. (Mrs R. M. Allison), *Biology Department, Canterbury University College, Christchurch, New Zealand*

1934 Oakley, Dr C. L., 58 *Cumberland Road, Bromley, Kent*
1930 Olive, G. W., C.B.E., *Dauntsey's School, West Lavington, near Devizes, Wilts*
1938 Oliver, James H., Ph.D., 16 *Southwark Street, London, S.E.* 1
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1939 Ommanney, F. D., Ph.D., *P.O. Box* 669, *Zanzibar, E. Africa*
1949 Orkin, P. A., *Natural History Department, The University, Aberdeen*
1947 Orr, A. P., D.Sc., *Marine Station, Keppel Pier, Millport, Isle of Cumbrae*
1910 Orton, Prof. J. H., D.Sc., F.R.S., *Sunny Bank, Burton, Wirral, Cheshire* (**Council**, 1945–48)
1945 Otago University Library, *Dunedin, N.* 1, *New Zealand*
*1928 Otter, G. W., *Southwood, Broadstone, Dorset*

Owen, G., *Department of Zoology, The University, Glasgow, W. 2*
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Pacific Biological Station, *Nanaimo, B.C., Canada*
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Pantin, C. F. A., Sc.D., F.R.S., *Trinity College, Cambridge.* (**Council,** 1930–32, 1933–36, 1937–40, 1941–44, 1945–49, 1951→ representing Cambridge University, 1946–49, 1951→)
Papworth, A. H., *The Terrace, Brixworth, Northampton*
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Pawlyn Bros., *Mevagissey, Cornwall*
Pawlyn, T. A., *Mevagissey, Cornwall*
Peacock and Buchan, Ltd., *Paint Manufacturers, Southampton*
Peel, Col. Sir Edward T., K.B.E., D.S.O., M.C., *c/o Messrs Peel and Co. Ltd., P.O. Box* 331, *Alexandria, Egypt.* (**Vice-President,** 1936→)
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Plymouth Education Authority, *Cobourg Street, Plymouth, Devon*
Plymouth, Port of, Incorporated Chamber of Commerce, *Plymouth, Devon*
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Plymouth Public Library, *Tavistock Road, Plymouth, Devon*
Pollard, D. G., 60 *Alcester Street, Stoke, Plymouth, Devon*
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Portsmouth Grammar School, *Biology Department, Portsmouth, Hants*
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Posner, Gerald S., *Marine Laboratory, University of Miami, Coral Gables* 34, *Florida, U.S.A.*
Potter, Miss E. A., *Westonbirt School, Tetbury, Glos*
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1950 Powell, R. R., 170 *Streatfield Road, Kenton, Middlesex*
1951 Pringle, I. G. St C., *Preston Hall, Uppingham, Rutland*
1939 Pringle, J. W. S., *Department of Zoology, Downing Street, Cambridge*
*1947 Pryor, Thomas, D.S.O., *Westbourne House, Saltash, Cornwall*
1939 Pumphrey, Prof. R. J., Ph.D., F.R.S., *Department of Zoology, The University Liverpool*
*1939 Purchon, Prof. R. D., Ph.D., *Department of Zoology, University of Malaya Singapore* 10

1936 Queen Mary College, Department of Zoology, *Mile End Road, London, E.*
1939 Queen's University, *Belfast, N. Ireland*
1947 Quekett Microscopical Club (W. Burrells, Hon. Sec.), 52 *Draycott Avenue Northwick Park, Harrow, Middlesex*
1932 Quick, H. E., F.R.C.S., 59 *Alexandra Road, Reading, Berks*
1952 Quiroga, Dr M., *Constitucion* 36, *Veracruz, Ver., Mexico*

1932 Ramalho, Dr A., *Instituto de Biologia Maritima, Cais do Sodré, Lisbon Portugal*
1949 Rasmussen, E., *The Zoological Laboratory, Landbohøjskolen, Bülowsvej Copenhagen V, Denmark*
1939 Rawlinson, Miss Ruth, 23A *Holbrook Road, Stoneygate, Leicester*
1947 Raymont, Prof. J. E. G., *Department of Zoology, The University, Southampto*
*1933 Rayner, G. W., *c/o C.S.I.R.O. Fisheries Division, Cronulla, N.S.W., Australi*
1949 Rees, C. B., D.Sc., *Oceanographic Laboratory*, 78 *Craighall Road, Edinburgh,*
*1936 Rees, W. J., D.Sc., 37 *Kings Road, Richmond, Surrey*
1928 Reid, D. M., *The Science Schools, Harrow-on-the-Hill, Middlesex*
1951 Reish, Donald J., *Allan Hancock Foundation, University of Southern California, Los Angeles, California, U.S.A.*
1935 Renouf, Prof. L. P. W., *University College, Cork, Eire*
1950 Renton, K., 55 *Tavistock Road, Stoke, Plymouth, Devon*
1940 Richards, P. B., *c/o Lloyds Bank Ltd., Truro, Cornwall*
1951 Rickard, Miss B., 68 *Furze Hill Road, Mutley, Plymouth, Devon*
1946 Rifaat, Ahmed, *c/o Marine Laboratory, Fort Kait Bey, Alexandria, Egypt*
1951 Rigby, Miss J. E., 184 *Walm Lane, London, N.W.* 2
1952 Rijal, Z., 36 *Great Cumberland Place, London, W.* 1
*1919 Ritchie, Prof. A. D., *Old College, The University, Edinburgh.* (**Council**, 1929–32, 1934–37)
1944 Ritchie, Prof. James, C.B.E., D.Sc., *Department of Zoology, The University Edinburgh.* (**Council**, 1944–47)
1948 Robinson, G. A., *Hilton Street, Over, Cambridge*
1949 Rodhe, Dr W., *Institute of Limnology, Uppsala, Sweden*
1951 Roe, W. D., *Fisheries Laboratory, Hamilton Dock, Lowestoft, Suffolk*
1940 Rogers, Dr T. Howard, *Naval Research Establishment, Halifax, Nova Scoti*
1952 Rosa, Horatio, *c/o Fisheries Division, F.A.O. of U.N., Vialle delle Terme d Caracalla, Rome, Italy*
1939 Ross, D. M., Ph.D., *Department of Zoology, University College, Gower Street London, W.C.* 1
1939 Ross, Miss Sybil S., 10 *Midmar Gardens, Edinburgh* 10
*1931 Rothschild, Rt Hon. Lord, G.M., Ph.D., *Merton Hall, Cambridge.* (**Council** 1939–42)
*1932 Rothschild, The Hon. Miriam (The Hon. Mrs George Lane), *The Cottage Ashton Wold, Peterborough, Northants.* (**Council**, 1942–45)

Rowe, Rev. A. F. C., *The Vicarage, Kingskerswell, Newton Abbot, Devon*
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Russell, E. S., O.B.E., D.Sc., *The Orchard, Beauharrow Road, St Leonards-on-Sea, Sussex*
(**Council**, 1927–30, 1932–35, 1936–39, 1940–43; **Vice-President**, 1948→)
Russell, F. S., D.S.C., D.F.C., F.R.S., *The Laboratory, Citadel Hill, Plymouth, Devon*. (**Council**, 1945→; **Secretary**, 1945→)
Russell, S. M. M., *Lincoln College, Oxford*

Sagay, A. A. E., *Warri College, Warri, Nigeria*
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Salisbury, Sir Edward J., Kt., C.B.E., D.Sc., Sec. R.S., 49 *Kew Green, Kew Gardens, Surrey*. (**Council**, 1934–37, 1940–43; **Vice-President**, 1948→)
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Smith, Admiral Sir Aubrey, K.B.E., C.B., M.V.O., *Hay's Wharf and Dock, Southwark, London, S.E.* 1. (**Council**, representing Fishmongers' Company, 1937–46, **Vice-President**, 1948→)
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1932 Smith, Prof. J. E., Ph.D., *Department of Zoology, Queen Mary College, M*
End Road, London, E. 1. (**Council**, 1943–46, 1948–51, representing Cam
bridge University 1949–51)
1939 Smith, S., Ph.D., *St Catharine's College, Cambridge*
1929 South Africa, Fisheries Division of, *Beach Road, Sea Point, Cape Tow*
S. Africa
1943 Southampton, The University
1945 Sparrow, B. W., *Corrofell, Newton Ferrers, Devon*
1934 Spaul, Prof. E. A., D.Sc., Ph.D., *Department of Zoology, The University, Lee*
1951 Spencer, C. P., Ph.D., *Marine Biological Station, University College*
N. Wales, Bangor, Caern
1930 Spooner, G. M., M.B.E., *The Laboratory, Citadel Hill, Plymouth, Devon*
1938 Spooner, Mrs G. M., Ph.D., *Fiveoaks, Crapstone, Yelverton, Devon*
*1939 Sproston, Miss Nora G., *Zoological Institute, Academia Sinica*, 320 *Yo Ya*
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1939 Stanbury, Miss F. A., *Biology Department, Plymouth Technical Colleg*
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1951 Stayte, D. J., 3 *Shears Road, Fairwater, Cardiff*
1925 Stephen, A. C., D.Sc., *Royal Scottish Museum, Edinburgh*
1951 Stephenson, R. M., *Somewhere, St Mary's, Isles of Scilly, Cornwall*
1930 Stephenson, Prof. T. A., D.Sc., F.R.S., *Department of Zoology, Universi*
College of Wales, Cambrian Street, Aberystwyth, Cardigan. (**Counc**
1941–44)
*1949 Stephenson, W., Ph.D., *Department of Zoology, University of Queenslan*
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1951 Steven, D. H., *St Cyprians, Yelverton, Devon*
1950 Steven, G. A., F.R.S.E., *St Cyprians, Yelverton, Devon*
1930 Steven, Mrs G. A., *St Cyprians, Yelverton, Devon*
1935 Stewart-Evison, Chas., *Manor East Lodge, Sidbury, Sidmouth, Devon*
1947 Stobart, T. R., *Warren House, Dartington Hall, Totnes, Devon*
*1934 Stoneman, Eric, *Zoology Department, The University, Reading*
1950 Street, P. A. R., 6 *Bellfield Avenue, Harrow Weald, Middlesex*
1936 Stubbings, H. G., 13 *Third Avenue, Denvilles, Havant, Hants*
1935 Sturdy, Mrs R. S., *Woodpiece, Apsley Guise, Bletchley, Bucks*
1946 Subrahmanyan, R., Ph.D., 52 *Choolai High Road, Madras 7, India*
1950 Sutcliffe, W. H. Jr, Ph.D., *Bermuda Biological Station, St George's We*
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1949 Sutton, Miss M. F., Ph.D., 6 *Effingham Lodge, Surbiton Crescent, Kingsto*
on-Thames, Surrey

1948 Tait, R. V., 62 *Nightingale Road, Rickmansworth, Herts*
1949 Talice, Prof. R. V., *Uruguayan Embassy*, 2 *Victoria Street, London, S.W*
1950 Taylor, A. R., A.M.I.N.A., 51 *London Road South, Lowestoft, Suffolk*
1932 Taylor, Prof. Sir Geoffrey I., D.Sc., F.R.S., *Farmfield, Huntingdon Ro*
Cambridge. (**Council**, 1932–35)
*1922 Taylor, Joseph A., 22 *Eaton Place, Brighton 7, Sussex*
1942 Templeman, Dr Wilfred, Director, *Newfoundland Fisheries Research Stati*
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1939 Thomas, I. M., *Department of Zoology, The University, Adelaide, S. Austra*
1950 Thomas, Miss J. G., Ph.D., *Royal Holloway College, Englefield Gre*
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1947 Thompson, J. A., *Dove Marine Laboratory, Cullercoats, Northumberland*

Torquay Natural History Society, *The Museum, Torquay, Devon*
Totton, A. K., M.C., *British Museum (Natural History), Cromwell Road, London, S.W. 7*
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IE SETTLEMENT OF *OPHELIA BICORNIS* SAVIGNY LARVAE

THE 1951 EXPERIMENTS

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INTRODUCTION

vestigation into the settlement and metamorphosis of *Ophelia bicornis* ıy larvae was begun in 1946 and has been continued during the breeding of each succeeding year. The results for the years 1946–50 inclusive lready been published (Wilson, 1948, 1951,[1] and 1952). In the last of three papers it was shown that size and shape of sand grains are less tant factors than had at first seemed probable, and that it appears that rvae react mainly to some other factor or factors. The nature of the was still largely speculative, but the evidence obtained pointed to ssibility that the larvae are influenced by minute amounts of organic als, dead or alive, present on the surface of the sand grains, and that etect them only on contact. Some of this organic material, particularly n the grains of most Salthouse Lake[2] sands, is repellent to them, and n sands *Ophelia* larvae do not settle. They settle in sands, of suitable which are not repellent, or only slightly so. The repellent property s to be largely, but not entirely, correlated with floatability of the sand on water, for repellent sands generally float readily when sprinkled dry the surface of water, while most non-repellent sands at once break h the surface film and sink. This phenomenon and related matters are sed in a preceding paper (Wilson, 1952).

oughout almost all the forty experiments with settling *Ophelia* larvae recorded in detail, it has been usual to wash and to sterilize—by heating ew minutes in tap water on a water-bath at about 100° C.—the various to be tested. In any one experiment all were treated alike in this way. ny sand samples so treated very good settlements had been obtained; rs smaller settlements or none at all. Results had been very consistent, lly the contrast between Bullhill Bank sand in which larvae readily orphosed and Salthouse Lake (Sts. I and II) sands, in which only a very

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s paper was delivered orally at a colloquium held in Paris in February 1950, and the given in it are abstracts from experiments recorded in greater detail in the 1952 paper, not additional to the latter.

a full description of the locality in the Exe estuary, with which this investigation is ly concerned, see the preceding 1952 paper, and for a photograph see the 1951 paper.

few larvae would do so, except after special treatments. These experimer were at first carried out in small flat-bottomed dishes in which the test sands, one kind to each dish, completely or almost completely covered t bottom, and later were carried out in conical vessels in which a smal quantity of sand could be effective. It is important to note that cylinders black paper ensured that during the daytime the vessels were illuminated or from above, the larvae (which are negatively phototactic for at least part the time) being thereby guided, if not driven, downwards towards the sanc From definitely repellent sands they would subsequently almost all cor away and swim up to attach themselves to the surface film of the water. Sor would often come away even from sands which gave good settlements. Sor experiments in which two or more sands were tested in the same dish show that larvae would settle and metamorphose in greatest numbers in their ov sterilized Bullhill sand in preference to others. While, however, there remain any suspicion of substances emanating from the Bullhill or other san inducing or inhibiting metamorphosis at a distance (as for Jägerstein's *Pro drilus*), experiments with more than one sand in the same dish could not employed generally, for they would involve uncertainties from which expe ments with only one sand-sample per dish were free. But when it had becor quite certain that *Ophelia* larvae are indifferent to the presence of a sa except when in actual contact with it, there remained no objection to testi different sands together in the same dish; indeed there are certain advantag such as the elimination of the error due to variations in the numbers of larv put into separate dishes. Because directional movements of the larvae a influenced by light it is advisable to keep in the dark all dishes that conta more than one sand, during the time allowed the larvae to settle. Experien has shown that at room temperatures in June and July larvae were genera ready to settle on the fifth day after fertilization and should be given 2 da in which to do so. This makes it possible to plan and work as many expe ments as possible during the 6 weeks or so when good larvae can be obtain

Towards the end of the 1950 season some experiments in the use of u sterilized sands gave particularly interesting results. It was found (usi conical vessels) that not only did fresh, unwashed, unsterilized Bullhill Ba sand induce heavier settlements and readier metamorphosis than the sa sand after the normal sterilization technique, but that fresh unwashed Sa house Lake (Sts. I and II) sand itself induced heavy settlements and rea metamorphosis, particularly Station I sand. The latter was, however, infer to fresh Bullhill Bank sand and Station II sand was worse (Exp. 37, Wils 1952). Experiments in which larvae could choose between these sands in same dish (Exps. 39 and 40, Wilson, 1952) confirmed the superiority of fr Bullhill sand over fresh Salthouse Lake sands as a medium for bringing ab settlement, but the discovery that sands from both localities when fre unwashed and unsterilized bring about a heavier settlement than when wash terilized was so striking as to necessitate further investigation. This and problems not completely elucidated by the end of the 1950 season were as starting-points for a new series of tests in 1951, and it is these latter n are recorded in the present paper.

The 1951 Experiments

principles on which experiments are devised and the general methods are fully explained in a previous paper (Wilson, 1952). Only those ls which differed from earlier practice need be mentioned here.

nost all the experiments in 1951 were carried out in Pyrex crystallizing s, 7 cm. diameter and 5 cm. high. They were a little more than half filled filtered sea water. Small quantities of the sands to be tested were ted into heaps of about 0·75 cm. diameter at the base and a few centi- es apart. The maximum number of heaps in any one dish was five. ss stated to the contrary, the sands tested were unsterilized. After adding ming larvae the dishes were immediately covered and kept in darkness they were examined 2 days later. For counting, the whole of a heap of was removed with a pipette to the counting dish. Any metamorphosing etamorphosed worms left behind sticking to the glass under the heap recorded and the numbers added to those found in the sand itself. The ities of sand being small, complete counts of the larvae present could ly be obtained for each, though occasionally when they were very rous and time pressed the system of awarding plus signs (see Wilson, p. 65) was adopted where it would not introduce error. Before the were removed the general condition of each dish was noted, with records vae still swimming, or on the surface film, or on the glass bottom. s otherwise stated it may be assumed that all stages counted were al and healthy.

os. 41 and 42 were carried out in filtered Outside Water, but all suc- ng experiments were in filtered Celtic Sea water. This water was kindly ted by Mr G. A. Steven on a cruise to the Celtic Sea early in July 1951, vas stored at a low temperature until used.

Experiment 41[1]

he suggestion of Dr H. W. Harvey the effect was tested of treating sands alumina and kieselguhr for comparison with the results obtained with ted charcoal. The alumina and kieselguhr were, of course, washed out ap water, and the sands rinsed in sea water before testing. Unfortunately s experiment a few lumps of kieselguhr or alumina remained in the The result (Table II) is almost negative so far as any favourable action alumina and kieselguhr is concerned.

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[1] The experiment numbers are consecutive with those already published.

Experiment 42

Dish A. It has been shown (Wilson, 1952, Table II) that the mean grai size of Bullhill Bank sand decreases with depth, but sands from variou depths had not previously been tested. The grading of the sands tested i this dish are given in Table I. The result (Table III) shows that by far th heaviest settlement occurred in the surface sand, but it is doubtful if this wa related to grade; it seems more likely that other factors were involved.

Dish B. The sand collected for me at Goulven, near Roscoff (see my 195 paper, p. 63), in which *O. bicornis* occurs in small numbers, is very muc coarser than that of the Bullhill Bank (1952, Table IV). That from La Jolla California (kindly collected by Dr R. Phillips Dales) is finer and of angula particles. It is the home of *Thoracophelia mucronata* which lives in it in grea abundance (Fox, Crane & McConnaughey, 1948). In neither was there an settlement of note, but as both sands had been collected a year previousl this result is scarcely significant in view of the findings with stored Bullhi Bank sands (see Exp. 47c, below).

Experiment 43

Dish A. Certain previous experiments had given some indication, short o final proof, that large grains of Bullhill Bank sand are made less attractive b mixing for a time with small grains of Salthouse Lake sand, the latter bein removed by sieving before the former are tested. All sievings, both befor and after mixing, were made with acetone-cleaned 60- and 86-mesh boltin silks in sea water, the sands not having been dried at any stage. The contr sand was sieved to an extent equal with the mixed sands. The result show here (Table IV) again gives some indication that contact with Salthous grains has an adverse effect on Bullhill sand when mixed together in sea wate The results for the mixture in distilled water bears a different interpretatio (see Exp. 44D below).

Dish B. The effect of exposing, on the laboratory roof, Salthouse Lak sands to the weather was tested. Small quantities of the sands were containe in funnels lined with filter-paper; rain water could drain away. They wer wet with rain when removed, but had previously been baked in the sun. The were rinsed in sea water before testing. The exposure made the sands eve less acceptable to the larvae than was the unexposed sand.

Experiment 44

Dish A. A comparison was made between natural Bullhill Bank sand an the same sand mixed with an equal volume of Salthouse Lake (St. II) san The mixture was less acceptable than the natural Bullhill sand (Table V *sh B.* A comparison was made between Bullhill Bank sand, previously ed and dried, and the same sand shaken for 7 min. on acetone-cleaned ng silk (200-mesh). The unshaken sand showed an unusually high float- y (see Wilson, 1952, p. 94). Neither sand produced a settlement: it is n below (Exps. 47 A, 48 A) that under these free-choice conditions larvae ot readily settle in sand which has previously been dried.

sh C. Exposing Bullhill Bank sand to the weather, on the laboratory made it less attractive to the larvae.

sh D. Soaking Bullhill Bank sand in distilled water, or sterilizing it, e it, under these free-choice conditions, unacceptable to the larvae. How- , the same sand, soaked in distilled water and completely covering the om of the vessel (in this instance a glass tube surrounded by a collar of k paper and lit from above), induced a very good settlement. It is esting to note that the metamorphosis rate appears to have been less than e control sand in the dark.

Experiment 45

his was a repetition of some of the tests of dishes B and D of the previous riment. A conical vessel containing Bullhill Bank sand, sterilized by the al technique, was added. This vessel was lit by light from above during laytime. The larvae came from the fertilization which supplied Exp. 44; were 3 days older and showed more tendency to stick to glass surfaces other objects than they had 3 days previously.

sh B. Both the washed and dried Bullhill Bank sand and the same sand friction on silk showed (Table VI) a greater settlement than previously. ould be noted, however, that there were a number of metamorphosed or morphosing larvae on the clean glass between the sand heaps and some of e larvae may have crawled out of the heaps. On the other hand, in the finger-bowl containing larvae from the same fertilization some larvae on ottom (but not on the surface film) were metamorphosing and some had pleted the process, although the great majority were still unmetamor- ed. Thus some of the young worms in the sand heaps might even have led in. There seems no doubt that older larvae metamorphose more ly than do younger ones and are probably not so selective. It may be ficant that there were fewer in the sand agitated on silk than in the other .

sh D. Normal sterilization technique, under free-choice conditions, again ned fewer larvae than sand which had only been rinsed in sea water, but e conical vessel containing sterilized Bullhill Bank sand a very good ement was obtained. The unusually small number of larvae in the sand h had only been rinsed (compared with Exp. 44 D) may have been due to swimming and exploratory activity on the part of the older larvae. A fair number of unmetamorphosed larvae were on the surface film, and som attached themselves to the bottom of the dish. Only a few larvae wer swimming freely when the dish was examined. Of those on the bottom mos were in various metamorphosis stages.

Throughout this experiment all stages were normal and very healthy. Th weather had been very warm for several days and the temperature throughou had been decidedly high. Their relatively advanced age may have been partl responsible for the readiness with which larvae metamorphosed on a clea glass surface.

Experiment 46

Dishes A 1 *and A* 2 *and Conical Vessel AA*. In a previous treatment (Exp. 41 Salthouse Lake sand had not been completely freed from large particles o alumina. The experiment was repeated here, care being taken to remove, b sedimentation, all large particles before mixing with the sand, in tap water After 3–4 hr. all the alumina was completely washed away and the sand rinse in sea water. Two dishes were set up, one with a small heap in the centre, th other had in addition a heap of Bullhill sand. Some of the treated sand wa also tested in a conical vessel.

The treatment with alumina does not seem (Table VII) to have made th Salthouse Lake sand more favourable under free-choice conditions in th dark, but in a conical vessel lit from above a heavy settlement and high rate o metamorphosis took place. But a settlement almost as good occurred i Salthouse Lake sand not treated with alumina (see conical vessel CC 1 below) At best the alumina made the sand slightly more favourable.

A recount of dishes A 1 and A 2 on 22 July 1951 showed no significan change; the larvae had not when older gone into the alumina-treated Salthouse Lake sand, even when there was no other sand in the dish.

Dish B and Conical Vessel BB. Lead-free ballotini retained on an 86-mes bolting silk sieve (see Wilson, 1952, p. 96) and then cleaned with stron sulphuric acid were here compared with natural Bullhill Bank sand. Althoug they induced some settlement they were nothing like as favourable as th Bullhill sand. In the conical vessel BB, however, they brought about a heav settlement. In the ballotini the larvae while fairly healthy were not as health as in the Bullhill sand.

Dish C and Conical Vessels CC 1 *and CC* 2. These were further tests o Salthouse Lake sands exposed to the weather on the laboratory roof. In thi experiment the sands were removed for testing during a spell of dry sunn weather and stored in sea water overnight. The weathered sands were chose by fewer larvae than the unweathered, and even in a conical vessel experimen the settlement and metamorphosis rate in the weathered sand was less than i the unweathered. Once again natural Bullhill sand collected most larvae.

Experiment 47

sh A. This experiment was designed to see if under free-choice conditions ill Bank sand was improved by treating with activated charcoal in tap r, and also to test the effect of drying (in a warm oven) Bullhill Bank after previous washing. It is seen (Table VIII) that hardly any larvae ted the latter whereas activated charcoal treatment (on sand *not* previously) had no effect on their choice.

sh B. This experiment examines the effect on settlement of treating ouse Lake (St. II) sand with alumina and with activated charcoal. The was not dried before treatment; it had been kept in its natural moist in a stoppered jar. The result leaves no doubt that activated charcoal ment makes the sand very acceptable to the larvae whereas alumina s it only slightly more so. It is interesting to note that there was a heavier ment in the fresh Salthouse Lake (St. II) sand in this dish than in the Bullhill Bank sand in dish A.

sh C. This is a comparison of Bullhill Bank sands collected in different . The sands had been kept in glass-stoppered jars in their natural semi- tate ever since collection; they had not been washed or dried. There is no t that the sand collected not very long before the test was the most tive, but it is interesting to note that the sand of 1947 was better than the les of 1948 and 1949. A sample from the year 1950 was tested later . 51 B).

Experiment 48

h A. In Exp. 47 A the Bullhill Bank sand treated with activated charcoal been washed in tap water but not dried. It was shown here (Table IX) f the sand be washed and dried (in a warm oven) before treatment the ated charcoal improves it compared with dried untreated sand, but it ins much inferior to the natural sand which has not been washed and .

sh B. In Exp. 47 B the Salthouse Lake (St. II) sand treated with alumina with activated charcoal had not been dried after washing in tap water. effect of drying (in a warm oven) after washing was tested here. It is that while alumina had little, if any, effect, activated charcoal so much oved the dried sand that it gave a similar result to charcoal-treated sand had not been dried. The latter, in this experiment, did not produce as a settlement as in Exp. 47 B. In all dishes in Exp. 47 a rather large er of larvae were inserted, and in setting up the present experiment larvae had been used in all three dishes; this almost certainly ins the lower figures for settlement in comparable sands in the two riments.

cluded in the present dish was another test of lead-free ballotini. They proved better than the washed and dried Salthouse Lake sand, but the larvae in them were not as healthy as in the sand heaps, where all stages were very healthy.

Dish C. This was designed to test the effect of treating Bullhill Bank sands with hot concentrated H_2SO_4. It is seen that whereas the recently collected sand was less favourable after treatment than before, the 1948 sand was, if anything, slightly improved. All stages were very healthy in the untreated sands and in nearly as good condition in those which had been treated.

Experiment 49

Dish A. Here were tested mixtures of recently collected Bullhill sand with Bullhill sand stored since 1948 and with recently collected Salthouse Lake (St. II) sand, both before and after drying. The mixture with the 1948 sand had even more larvae in it than the 1951 sand alone (Table X), but many of these were unmetamorphosed and the metamorphosis rate was not as good as in the 1951 sand. As usual, the more significant figures are probably those for the metamorphosing and metamorphosed larvae, ignoring those for the unmetamorphosed. The 1948 sand here did better than in Exp. 47C, but there is always the possibility that samples of sand removed from a large jar, in which it has been stored undisturbed for a long time, vary in properties if bacterial or other growths or chemical actions have been taking place.

*Dish B and Conical Vessels BB*1 *and BB*2. This was mainly to test the effect of treating the Bullhill Bank sand, collected in 1948, with activated charcoal after washing with tap water (not dried). The results shows that under free-choice conditions the treatment brought about a slight improvement There was a very marked improvement in the conical vessel tests. It must be noted, however, that the figures for the untreated sand in conical vessel BB1 are not quite complete; a few more larvae of all stages were not counted (owing to lack of time). It was obvious, however, that there were not enough to double the figures, and the total for all stages would have been well under one hundred.

Experiment 50

In this experiment the bottom of each crystallizing dish was completely covered with Bullhill Bank sand, one with sand collected about a month previously, the other with the sand collected in 1948, which under free-choice conditions in Exp. 47C was not attractive to the larvae. A very large number of larvae were put into each dish and the dishes were kept in the dark for the usual 2 days allowed for settlement. Dish I, containing the recent sand, was examined first (Table XI). Almost all the larvae had disappeared into the sand and only a very few were still swimming and there were none on the

ce film. Small portions of the sand were removed from all parts of the
, and during a 20-min. search of these portions a number of larvae were
d, almost all of them metamorphosed. In the dish containing the 1948
there were, in contrast, a large number of larvae swimming freely and
nsiderable number, unmetamorphosed, attached to the surface film. A
in. search of small quantities of sand from all parts of this dish yielded
ively few larvae, and most of these were unmetamorphosed. The experi-
t was continued for another 2 days, by which time most of the larvae in
dish with 1948 sand had settled and metamorphosed.

his result with dishes in which the bottom is completely covered with
supports the contention that the larvae find their sand by chance contact.
ese dishes they could not easily miss it, as they can when only small heaps
employed. Under free-choice conditions, with small heaps of sand there
, after 2 days in the dark, always a large number of unmetamorphosed
e swimming freely or attached to the surface film. It is noteworthy that
the completely suitable sand the larvae settled straight away, whilst with
not so suitable sand there was some delay.

Experiment 51

his was the last experiment carried out in 1951. Unfortunately the larvae
were, for some unknown reason (not temperature), slow in their develop-
and the results are therefore not as definite as they might otherwise have
. This is so especially for dish A, the examination of which began at
.m. on the second day. When it was found that the larvae were still at
her early stage of development the examination of the other dishes was
poned for a few hours, but it was not possible to leave them until the
day.

ish A. This was intended as a test of different mesh sizes of relatively
h Bullhill Bank sand. Sieving was done in sea water through bolting silks
ch had been cleaned with acetone. As it has been shown that greater
ion with silk than that involved in simple sieving has only a little effect on
acceptability of the sands used it seems practicable to use silk sieves for
tests. The results (Table XII) appear to confirm this conclusion, and
also seem to show that the smallest mesh sizes do not attract as many
ae as does the natural sand, or as do the larger-sized grains. Unfor-
ately, for the reason already stated, the experiment is inconclusive. On the
le the larvae were healthy, but in the >60-mesh sand there were a
ber in poor condition.

ish B. Salthouse Lake (St. II) sand washed and soaked in aquarium water
several days is apparently not much altered in property so far as the larvae
concerned. On the other hand, the same sand kept with some living
lt *Ophelia* and exposed to their excretions and extruded genital products

was made even less attractive than before. All larval stages in this dish were healthy.

Dish C. This shows that Bullhill Bank sand collected in 1950 was, like those of earlier years (see Exp. 47), less acceptable to the larvae than that collected more recently. It may be noted that of the twenty-seven unmetamorphosed larvae recorded for the 1950 sand the majority swam away readily when disturbed, but in the 1951 sand the unmetamorphosed larvae had attached themselves more strongly to the grains and were probably beginning to metamorphose. In this dish all stages were very healthy.

Dish D. This was designed to investigate the effect of washing fresh Bullhill Bank sand in tap water, of treating it with soluble reagents (alcohol and formalin) which would kill living matter and which could then be removed by washing (in sea water). A sample of sand dried and heated to redness (which in early experiments had given good settlements) was also tested. The results show that washing in tap water for several minutes had little effect (unlike prolonged soaking in distilled water—see Exp. 44 D); the alcohol and formalin treatments made the sand disliked, while the sand heated to redness produced only a small settlement under these free-choice conditions and, as often in a heated sand, the larvae tended to be in poor condition and there were some dead. In all the other sands all stages were very healthy.

Dish E. This small dish had the bottom completely covered with lead-free ballotini of sizes between 60 and 86 mesh, the largest obtainable. During 2 days in the dark there was a good settlement. Although many larvae were still swimming freely, or were attached to the surface film, a considerably larger number were observed among the ballotini. Once again a dish with the bottom completely covered with 'sand' produced a bigger settlement than would have taken place in a small heap in the middle of an area of clean glass. All stages were healthy and normal.

THE 1951 RESULTS

It is now possible to extract from the experiments a series of data which can be arranged fairly logically. Except when otherwise stated, this series is based on the 1951 'free-choice' experiments, that is to say the experiments in which the sands were presented in small heaps on the bottoms of clean glass crystallizing dishes in the dark and in which the larvae were not forced into contact with them by negative phototaxis. This method has revealed that the larvae are capable of a finer discrimination between sands than was evident in most earlier experiments. Sands which induce settlement and metamorphosis will be referred to as 'attractive', and it will become apparent there are varying degrees of attractiveness.

(1) Sand from the surface of the Bullhill Bank, where *Ophelia bicornis* adults are very common, is very attractive to the larvae of that species, provided that

s been collected within recent weeks and kept in its natural moist state. ontact with it they quickly settle and metamorphose.

Larvae find the sand by chance contact, they are not attracted to it from tance by olfactory or other senses. This should be remembered when d is described as being attractive. It is only attractive to them while they n contact with it.

Nothing dissolves out of the sand to induce the metamorphosis of larvae n actual contact with it (the 1951 and all previous experiments).

Washing for a few minutes in tap water, followed by sea water, has little t on fresh Bullhill Bank sand, although it does seem to make it a little less ctive (Exp. 51 D).

The attractiveness of fresh Bullhill Bank sand is destroyed by soaking everal days in distilled water, but not by soaking in sea water (Exps. 43 A, If, however, the sand which has been soaked in distilled water com- ly covers the bottom of a vessel lit from above during the daytime, larvae in it and metamorphose readily (Exp. 44 D), though probably not as ly as they would do in fresh sand under like conditions.

The attractiveness of fresh Bullhill Bank sand is greatly reduced, or oyed altogether, by heating in tap water to about 100° C. (Exps. 44 D, This was the normal sterilization technique adopted for almost all riments in previous years. However, in a conical vessel lit from above g the daytime (as in earlier experiments) sand so treated will bring about vy settlement with a high rate of metamorphosis (Exp. 45 DD), though an r experiment (Exp. 37, Wilson, 1952) showed that the attractiveness of ized sand is less than that of fresh sand even in conical vessel experiments.

The attractiveness of fresh Bullhill Bank sand is greatly reduced or oyed altogether by drying in a warm oven after washing in tap water s. 44 B, 45 B, 47 A, 48 A).

The attractiveness of Bullhill Bank sand which has been reduced by ous washing and drying seems to be still further reduced, though only light extent, by friction dry on silk (Exps. 44 B, 45 B).

There is little or no difference in attractiveness between fresh Bullhill sand and the same sand agitated on silk in sea water (Exp. 51 A).

) The attractiveness of Bullhill Bank sand is reduced or destroyed by ng in sea water to which absolute alcohol or neutral formalin has been d (Exp. 51 D).

) The attractiveness of fresh Bullhill Bank sand washed quickly in dis- water and then treated with hot concentrated sulphuric acid is reduced ot entirely destroyed (Exp. 48 C) as it is when soaked for a long period in led water alone (Exps. 43 A, 44 D).

) Fresh Bullhill Bank sand which has been washed and dried has its ctiveness partially restored by treating in tap water with activated oal (Exp. 48 A).

(13) Fresh Bullhill Bank sand which has merely been washed before treatment with activated charcoal in tap water has an attractiveness similar to that of the untreated sand (Exp. 47A).

(14) The attractiveness of fresh Bullhill Bank sand is destroyed by exposure to the weather (Exp. 44C).

(15) The surface sand of the Bullhill Bank is considerably more attractive than that from deeper down (Exp. 42A).

(16) There is some indication that the smallest grains of fresh Bullhill Bank sand are not as attractive as are the larger ones (Exp. 51A).

(17) The attractiveness of Bullhill Bank sand is greatly reduced by prolonged storage (Exps. 47C, 48C, 49A and B, 50, 51C).

(18) The attractiveness of a mixture of equal volumes of fresh Bullhill Bank sand and of Bullhill Bank sand stored for several years is similar to that of fresh Bullhill Bank sand alone, and is greater than that of the stored sand alone (Exp. 49A). This may indicate that while storage of the sand, without drying, makes it less likeable by the larvae it does not render it repellent to them. A similar conclusion seems to be suggested by the result of Exp. 50.

(19) The attractiveness of Bullhill Bank sand which has been reduced by prolonged storage is possibly to some extent restored by treatment with activated charcoal (Exp. 49B) or hot concentrated sulphuric acid (Exp. 48C). Previous experiments have shown that under conical vessel conditions the former treatment may be expected to improve favourable as well as unfavourable sands (Wilson, 1952).

(20) Bullhill Bank sand which has been heated to 900–1000° C., and which in conical vessel experiments brings about a heavy settlement and a good rate of metamorphosis (Wilson, 1952), is under free-choice conditions only slightly attractive (Exp. 51D).

(21) The attractiveness of fresh >60-mesh Bullhill Bank grains is reduced after having been mixed for several days in sea water with fresh <86-mesh Salthouse Lake (St. II) grains (Exp. 43A).

(22) The attractiveness of a mixture of equal volumes of fresh Bullhill Bank sand and fresh Salthouse Lake (St. II) sand is less than that of fresh Bullhill Bank sand alone (Exps. 44A, 49A, and compare paragraph 18 above). If the Salthouse sand be washed and dried before mixing with the fresh Bullhill sand the mixture is almost completely unattractive, indicating that the drying of the Salthouse sand has made it repellent (Exp. 49A, and compare Exp. 48B with Exps. 43B, 46C, 47B, 51B).

(23) Fresh Salthouse Lake (St. II) sand is much less attractive than fresh Bullhill Bank sand (Exps. 43B, 46C). This relation is not altered by previously washing both sands with tap water (Exp. 41). [N.B. Fresh Salthouse Lake (St. II) sand is silty.]

(24) The slight attractiveness of Salthouse Lake (St. II) sand washed in tap water is not significantly altered by treatment in tap water with alumina

th kieselguhr (Exps. 41, 46 A 1 and A 2), though there is some evidence after alumina the attractiveness may be slightly increased (Exp. 47 B and pare Exp. 46 AA with Exp. 46 CC 1). Treatment with alumina of the same after previous drying had no significant effect (Exp. 48 B).

) Salthouse Lake (St. II) sand which has not been dried is made strongly tive by treatment, in tap water, with activated charcoal (Exp. 47 B). same sand made strongly repellent by previous drying (see paragraph 22 e) is also made strongly attractive, and apparently to about the same t, by activated charcoal treatment (Exp. 48 B).

5) The exposure of Salthouse Lake (St. II) sand to the weather for al weeks lessens its natural slight attractiveness (Exps. 43 B, 46 C and pare Exp. 46 CC 2 with Exp. 46 CC 1). [N.B. Such exposure involves drying, ell as washing by rain.]

) The slight attractiveness of fresh Salthouse Lake (St. II) sand is ly altered by soaking for several days in aquarium water (Exp. 51 B). ttractiveness is, however, reduced, after contact (in aquarium water for al days) with *Ophelia* adults, their excretions and genital products . 51 B).

) Coarse gravel, inhabited by *Ophelia bicornis* to the extent of 12–15 per . collected one year previously from Goulven, near Roscoff, and washed p water before testing, is almost completely unattractive (Exp. 42 B, and pare paragraph 17 above).

) Fine, gritty sand from La Jolla, California, inhabited by the allied *acophelia mucronata*, collected one year previously and washed in tap r before testing, is almost completely unattractive (Exp. 42 B and compare graph 17 above).

) Acid-cleaned, lead-free ballotini > 86-mesh (mainly < 60-mesh) are erately attractive (Exp. 46 B) and are more attractive than Salthouse Lake II) sand which has been dried (Exp. 48 B). In a conical vessel, and when pletely covering the bottom of a dish, these ballotini induce a good settle- t and a good rate of metamorphosis (Exps. 46 BB, 51 E).

1) Some sands which are relatively unattractive under free-choice con- ns will, in conical or flat-bottomed vessels, where the bottom is com- ly covered, induce good and sometimes heavy settlements and good morphosis rates, although likely to be delayed when compared with rongly attractive sand under the same conditions (Exps. 44 D, 45 DD, , CC 1 and CC 2, 50, and many experiments in previous years).

Discussion

n the results it appears that sands fall roughly into three classes according he reactions of the larvae towards them. There are, first, *attractive* ls which induce heavy settlements, with almost immediate metamorphosis, in both free-choice and conical vessel or ordinary dish experiments. Secondly there are sands which under free-choice conditions retain very few larvae and in which metamorphosis does not take place readily. Such sands are fairly attractive in conical vessels, or in dish experiments where the bottom is completely covered by them and which during the day are lit from above. Although all the larvae in the vessels or dishes do not enter them, these sands under such conditions usually induce good settlements and most larvae metamorphose reasonably soon after entering, but they are not as attractive as are sands of the first class. These second-class sands can be regarded as *neutral*, being neither particularly attractive nor particularly repellent to larvae in contact with them. In the third or last class are sands in which few or no larvae will settle and metamorphose under either experimental conditions; such sands can be regarded as being *repellent* to the larvae. As is only to be expected, the classes grade into one another and there are many sands which occupy intermediate positions. The relative positions of several sands are compared in Tables XIII–XV. These are based primarily on the free-choice experiments of 1951, but some results from conical vessel and ordinary dish experiments of earlier years are inserted in italics. This is done by assuming that Bullhill Bank sand sterilized in the normal way is neutral. With it are then compared results with other sands in the same experiments. It is easy to see whether a sand is better or worse and to obtain a fair idea of how far it departs from neutrality.

It will be observed (Table XIII) that the only fully attractive sand is fresh Bullhill Bank surface sand and that its attractiveness is lessened if it be subject to almost any treatment. Brief washings in tap water, followed or not by activated charcoal treatment in tap water for half an hour, and agitating on silk in sea water, have little or no effect on attractiveness, but normal sterilization, drying (after washing) at low heat, and soaking for a prolonged period in distilled water all render the sand neutral. Sand which has merely been stored (naturally moist) for several years occupies a variable intermediate position. Certain treatments, particularly activated charcoal treatment, impart some degree of attractiveness to Bullhill Bank sands which would otherwise be neutral. Some of these are treatments which could be expected to free the sand from adhering organic matter.

Salthouse Lake (Sts. I and II) sands (Table XIV) when fresh are not quite neutral but are almost so. It is practically certain that different samples vary in the slight degree of attractiveness they show when fresh and that St. II sand is always less attractive than St. I. When these sands are dried at a low temperature, or undergo normal sterilization technique, or are variously treated, they become strongly repellent. As exposure to the weather will also make them repellent it is possible that sometimes in nature they can be obnoxious to the larvae. Certain treatments, again particularly activated charcoal and those which can be expected to remove organic matter, will e the sand better from the larval point of view. In this connexion it is esting to note (Table XV) that the perfectly clean ballotini seem to py a position half-way between neutral and fully attractive sands, and might be even more attractive if the constituents of the glass were pletely insoluble so that all poisoning effects were eliminated, and if the -free ballotini were not so close to the lower limit of grade which the e can penetrate.

f the three mixtures (see Exps. 44A and 49A) tested under free-choice itions it is interesting to note (Table XV) that a fifty–fifty mixture of ly attractive with a partially attractive sand (stored Bullhill Bank sand) st as effective as a fully attractive sand alone. A mixture of a nearly ral, slightly attractive sand (fresh Salthouse Lake, St. II) with a fully ctive sand is moderately attractive, while a mixture of a fully attractive with a fully repellent sand (dried Salthouse Lake, St. II) is either neutral mewhat repellent. From the tables it should be possible to forecast with accuracy the settlements which will be obtained in mixtures of various s.

erhaps the most significant results are those which show that sterilization fresh sand, or any treatment which could be expected to kill living nisms, have most effect in rendering a sand less attractive that it was re. Fully attractive sands seem to become neutral, while sands that are ly neutral when fresh become repellent. On the sand grains there is a and fauna of bacteria and other micro-organisms, and it is possible that when dead are objectionable to the *Ophelia* larvae, but are attractive, or y rate not repellent, when alive. The sand grains are likely also to carry ngs of organic materials which are changed in chemical and physical acter by sterilization and other treatments. Bullhill Bank sands may differ those of the Salthouse Lake (Sts. I and II) not only in the number and ies of micro-organisms present but also in the quantity and quality of the -living organic matter adhering to the sand grains.

may be significant that treatments (such as hot concentrated sulphuric) which would remove organic matter make repellent sands more favour- and they then attract a settlement similar to that of the clean ballotini. n this it would appear that larvae will settle in some force in perfectly n sands of suitable grade, but more strongly still if the right kind and ntity of living organisms or organic coatings, or both, are present. In h Bullhill Bank sands the right kinds and quantities are present, but the vourable Salthouse Lake sands have the wrong kinds or quantities.

he effect on larval settlement of sterilization has been shown not only by own experiments in 1950 and 1951, but also by some work on the settle- t of the polychaete *Pygospio elegans* Clap. carried out by Smidt (1951). summarizes (p. 62) his own results as follows. 'A natural substratum d and mud) stimulates metamorphosis, while the lack of a substratum will impede it. Further, pure sterile sand and naturally occurring pebbles have an impeding effect. As naturally occurring Wadden sand is always mixed with some mud particles and organic particles, it is possibly the presence of these which is of importance for the settling and metamorphosis of the larvae. Larvae kept without any substratum had not yet metamorphosed after two months.' His sterile sand had been strongly heated and treated with acid. The sands were presented in wine glasses with pointed bottoms (equivalent to my conical vessels made from funnels). The percentage settlement obtained in the sterile sand was very small and was probably considerably less than *Ophelia* larvae give in acid-cleaned sands. In this connexion it should be remembered that *Ophelia* when adult lives in much cleaner sands that does *Pygospio*, and this difference may be a reflexion of that habit. Smidt does not carry his observations further and does not comment on the possible role of bacteria or of adhering organic matter.

It is, of course, known that on solid surfaces bacterial and other films are often necessary before any great settlement of larger sedentary species occurs (for a brief summary of the literature see Wilson, 1952); it would not be surprising, therefore, if much the same sort of thing were to prove true of species settling in sands and muds.

Some of my earlier conclusions (Wilson, 1952) concerning adsorbed organic matter on sand grains and its connexion, among other things, with floatation properties of the dried grains, receive support from some recent work which was unknown to me until after my own paper had been written. Turmel (1950), from observations concerning variation in the rate of percolation of water in dune sands of different kinds, and from observations on floatability of the sands when sprinkled dry on to water, came to a similar conclusion. He maintains that the grains of floatable (non-wettable) dune sands are covered with an extremely thin film of organic matter. In experiments similar to my own (Wilson, 1952) he showed that after treatment with the fat solvents alcohol, ether, carbon disulphide and tetrabenthene, grains of floatable sands did not become more readily wetted, but after treatment with hydrogen peroxide they became completely wettable. He considered that his experiments demonstrated that non-wettability was due to a thin coating of organic matter. The nature of this organic film, however, had still to be determined.

SUMMARY

Further tests on the settlement reactions of *Ophelia bicornis* larvae have shown that both Bullhill Bank and Salthouse Lake sands are less favourable to the larvae after sterilization than they are when fresh and untreated. The effect of a large variety of treatments of both sands has been investigated under conditions where the larvae were free to choose between two or more samples of sands presented together in the same dish. Results from these experiments,

in conjunction with results obtained using conical vessels, show that
s may be classed as *attractive*, *neutral* or *repellent* with intermediate
ing between these three main categories. It is concluded that organic
rial, living or dead, on the sand grains plays an important role in
ering a sand attractive or repellent to the larvae.

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TABLE I. BULLHILL BANK SAND COLLECTED 19 JUNE 1951

(From the middle of the bank; *Ophelia* present in abundance.)
Percentage mesh sizes by weight, after washing away silt.

(6) >26 mesh	(5) 26–40 mesh	(4) 40–60 mesh	(3) 60–86 mesh	(2) 86–100 mesh	(1) 100–200 mesh	Silt	Mean category	Percentage floatability
0·0	1·4	29·6	61·0	7·3	0·7	Slight	3·24	30–40
0·1	0·3	13·9	66·7	16·3	2·8	A little	2·93	*ca* 1
1·1	1·8	23·3	53·5	18·0	2·4	A little	3·07	*ca* 1
0·2	0·6	20·3	65·1	11·3	2·3	A little	3·06	*ca* 1

et)

TABLE II. EXPERIMENT 41

(Begun 25. vi. 51 with larvae from a fertilization of 20. vi. 51.)
Result on 27. vi. 51.

	Bullhill Bank sand (19. vi. 51) washed in tap water (1)	Salthouse Lake sand (St. II—19. vi. 51) washed in tap water (2)	Sand as (2) mixed with alumina since 21. iii. 51 (3)	Sand as (2) mixed with kieselguhr since 21. iii. 51 (4)
Metd	24	0	0	0
Meting	17	1	0	1
Unmet.	5	13	34	18
Total	46	14	34	19

d = metamorphosed; meting = metamorphosing; unmet. = unmetamorphosed; *d* = dead.

TABLE III. EXPERIMENT 42

(Begun 26. vi. 51 with larvae from a fertilization of 21. vi. 51.)

Result on 28. vi. 51.

Dish A. Bullhill Bank sands collected 19. vi. 51, *unwashed*

	Sand from surface	Sand from 6 in. below surface	Sand from 12 in. below surface	Sand from 18 in. below surface
Metd } Meting }	232	60	9	34
		5	2	10
Unmet.	15	11	12	8
Total	247	76	23	52

Dish B

	Bullhill Bank sand (19. vi. 51) washed in tap water	Sand from La Jolla, California, washed in tap water	Sand from Goulven, near Roscoff, washed in tap water
Metd	108	0	0
Meting	18	3	0
Unmet.	10	8	14
Total	136	11	14

TABLE IV. EXPERIMENT 43

(Begun 11. vii. 51 with larvae from a fertilization of 6. vii. 51.)

Result on 13. vii. 51.

Dish A

	Bullhill sand (19. vi. 51) > 60-mesh in sea water for several days	Bullhill sand > 60 mesh mixed in sea water with < 86-mesh S.L. (St. II) for several days	Bullhill sand > 60-mesh in distilled water for several days	Bullhill sand > 60-mesh mixed in distilled water with < 86-mesh S.I (St. II) for severa days
Metd	56	33	0	0
Meting	20	10	0	0
Unmet.	7	4	0	1
Total	83	47	0	1

Dish B

	Bullhill sand (19. vi. 51) rinsed in sea water	Salthouse Lake (St. II—19. vi. 51) sand rinsed in sea water	Salthouse Lake (St. II—19. vi. 51) exposed to weather for 3 weeks, rinsed in sea water	Salthouse Lake (St. I—19. vi. 51) exposed to weathe for 3 weeks, rinse in sea water
Metd	43	3	1	0
Meting	13	2	1	0
Unmet.	7	11	2	5
Total	63	16	4	5

TABLE V. EXPERIMENT 44

(Begun 15. vii. 51 with larvae from a fertilization of 10. vii. 51.)

Result on 17. vii. 51.

Dish A

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Mixture of about equal volumes of Bullhill (19. vi. 51) and Salthouse Lake (St. II—19. vi. 51) sands rinsed in sea water
Metd	19	8
Meting	4	5
Unmet.	3	0
Total	26	13

Dish B

	Bullhill Bank sand (19. vi. 51) washed and dried. (Floatability 70–80 %)	Bullhill Bank sand (19. vi. 51) washed and dried. Shaken on silk for 7 min. (Floatability 95 %.)
Metd	0	0
Meting	0	0
Unmet.	5	1
Total	5	1

Dish C

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51) exposed to weather for 3½ weeks, rinsed in sea water
Metd	4	0
Meting	0	0
Unmet.	11	4
Total	15	4

Dish D

	Bullhill Bank sand (19. vi. 51) soaked in sea water for 2 days	Bullhill Bank sand (19. vi. 51) soaked in distilled water for 2 days	Bullhill Bank sand (19. vi. 51), normal sterilization technique
Metd	164	0	0
Meting		0	0
Unmet.	1	1	0
Total	165	1	0

As above, but in small glass-stoppered tube

Metd	17	+ +
Meting	34	
Unmet.	12	

TABLE VI. EXPERIMENT 45

(Begun 18. vii. 51 with larvae from a fertilization of 10. vii. 51.)

Result on 21. vi. 51.

Dish B

	Bullhill Bank sand (19. vi. 51) washed and dried. (Floatability 70–80 %)	Bullhill Bank sand (19. vi. 51) washed and dried. Shaken on silk for 8 min. (Floatability 100 %.)
Metd	11	3
Meting	3	2
Unmet.	4	4
Total	18	9

	Dish D		*Conical vessel DD*	
	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51), normal sterilization technique	Bullhill Bank sand (19. vi. 51), normal sterilization technique	
Metd	3	0	33	+
Meting	5	4	7	
Unmet.	0	6	3	
Total	8	10	*c.* 90	

TABLE VII. EXPERIMENT 46

(Begun 17. vii. 51 with larvae from a fertilization of 12. vii. 51.)

Result on 19. vii. 51.

	Dish A 1 Bullhill Bank sand (19. vi. 51) rinsed in sea water	*Dish A 1* Salthouse Lake (St. II—19. vi. 51) treated with alumina	*Dish A 2* Salthouse Lake (St. II—19. vi. 51) treated with alumina	*Conical vessel AA* Salthouse Lake (St. II—19. vi. 51) treated with alumina
Metd	14	3	1	15
Meting	13	1	1	8 } +++
Unmet.	4	1	1	1
Total	31	5	3	c. 150–200

	Dish B. Bullhill Bank sand (19. vi. 51) rinsed in sea water	*Dish B.* Lead-free ballotini > 86-mesh, acid cleaned	*Conical vessel BB.* Lead-free ballotini > 86-mesh, acid cleaned
Metd	14	15	8
Meting	43 } ++	15	12 } +++
Unmet.	2	5	9
Total	c. 150–200	35	c. 150–200

	Dish C Bullhill Bank sand (19. vi. 51) rinsed in sea water	*Dish C* Salthouse Lake (St. II—19. vi. 51) stored in sea water overnight	*Dish C* Salthouse Lake (St. II—19. vi. 51) exposed to weather for 4 weeks, stored in sea water overnight	*Dish C* Salthouse Lake (St. I—19. vi. 51) exposed to weather for 4 weeks, stored in sea water overnight	*Conical vessel CC 1* Salthouse Lake (St. II—19. vi. 51) stored in sea water overnight	*Conical vessel CC 2* Salthouse Lake (St. II—19. vi. 51) exposed to weather for 4 weeks, stored in sea water overnight
Metd	7	6	0	0	14	1
Meting	35 } +	3	0	1	5 } ++	3 } +
Unmet.	4	1	2	0	5	16
Total	c. 100	10	2	1	c. 75	c. 40

TABLE VIII. EXPERIMENT 47

(Begun 23. vii. 51 with larvae from a fertilization of 18. vii. 51.)

Result on 25. vii. 51.

Dish A

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51) washed in tap water, treated with activated charcoal (in tap water for half an hour) washed in tap water, then in sea water	Bullhill Bank sand (19. vi. 51) washed in tap water and dried. Rinsed in sea water
Metd	37	35	1
Meting	4 ++	7 ++	0
Umet.	1	9	7
Total	c. 150–200	c. 150–200	8

Dish B

	Salthouse Lake sand (St. II—19. vi. 51) rinsed in sea water	Salthouse Lake sand (St. II—19. vi. 51) treated with alumina in tap water, washed in tap water, then in sea water	Salthouse Lake sand (St. II—19. vi. 51) treated with activated charcoal in tap water, washed in tap water, then in sea water
Metd	30	38	16
Meting	6	9	34 ++
Unmet.	16	17	c. 60
Total	52	64	c. 300–350

Dish C

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (1947) rinsed in sea water	Bullhill Bank sand (1948) rinsed in sea water	Bullhill Bank sand (1949) rinsed in sea water
Metd	35	21	0	2
Meting	11 +	6	3	1
Unmet.	7	11	8	6
Total	c. 100–150	38	11	

TABLE IX. EXPERIMENT 48

(Begun 26. vii. 51 with larvae from a fertilization of 21. vii. 51.)

Result on 28. vii. 51.

Dish A

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51) washed in tap water and dried. Treated with activated charcoal in tap water, washed in tap water, then in sea water	Bullhill Bank sand (19. vi. 51) washed in tap water and dried. Rinsed in sea water
Metd	57	6	0
Meting	12	12	0
Unmet.	5	14	4
Total	74	32	4

Dish B

	Salthouse Lake sand (St. II—19. vi. 51) washed in tap water and dried. Rinsed in sea water	Salthouse Lake sand (St. II—19. vi. 51) washed in tap water and dried. Treated with alumina in tap water, washed in tap water, then in sea water	Salthouse Lake sand (St. II—19. vi. 51) washed in tap water and dried. Treated with activated charcoal, washed in tap water, then in sea water	Salthouse Lake sand (St. II—19. vi. 51) washed in tap water, treated with activated charcoal, washed in tap water, then in sea water	Lead-free Ballotini > 86 mesh, acid-cleaned, washed in tap water, then in sea water
Metd	0	0	10	8	0
Meting	0	2	20	32	10
Unmet.	2	3	29	17	15 *d* 12
Total	2	5	59	57	37

Dish C

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51) washed in distilled water, treated with hot conc. H_2SO_4, washed in distilled water, then in sea water	Bullhill Bank sand (1948) rinsed in sea water	Bullhill Bank sand (1948) washed in distilled water, treated with hot conc. H_2SO_4, washed in distilled water, then in sea water
Metd	51	5	2	3
Meting	8	2	2	4
Unmet.	3	10	4	18
Total	62	17	8	25

TABLE X. EXPERIMENT 49

(Begun 28. vii. 51 with larvae from a fertilization of 23. vii. 51.)

Result on 30. vii. 51.

Dish A

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (1948) rinsed in sea water	Bullhill Bank sands of 19. vi. 51 and 1948 mixed in equal proportions, rinsed in sea water	Bullhill Bank (19. vi. 51) and Salthouse Lake (St. II—19. vi. 51) sands mixed in equal proportions, rinsed in sea water	Bullhill Bank sand (19. vi. 51) rinsed in sea water mixed with equal volume of Salthouse Lake sand (St. II—19. vi. 51), washed and dried
Metd	23	8	15	8	0
Meting	14	8	22	14	1
Unmet.	7	28	30	25	8
Total	44	44	67	47	9

	Dish B			*Conical vessel BB 1*	*Conical vessel BB 2*
	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (1948) rinsed in sea water	Bullhill Bank sand (1948) treated in tap water with activated charcoal, washed in tap water, then in sea water	Bullhill Bank sand (1948) rinsed in sea water	Bullhill Bank sand (1948) treated in tap water with activated charcoal, washed in tap water, then in sea water
Metd	25	2	3	9	20
Meting	24	1	9	17	18 } +++
Unmet.	4	7	18	28	21
Total	53	10	30	54	*c.* 250–350

TABLE XI. EXPERIMENT 50

(Begun 28. vii. 51 with larvae from a fertilization of 23. vii. 51.)

	Dish I	*Dish II*
	Bottom of dish *completely covered* with Bullhill Bank sand (19. vi. 51), rinsed in sea water	Bottom of dish *completely covere* with Bullhill Bank sand (1948) rinsed in sea water
	Result on 30. vii. 51.	
On surface film	None	Considerable number unmet.
In mid-water	Two or three swimming	Large number swimming
In sand (small portion)	Metd 24 Meting 6 Unmet. 1	Metd 1 Meting 4 Unmet. 8
	Result on 1. viii. 51.	
On surface film	A very few unmet. or meting; 1 metd	A fair number unmet., a very f meting or metd
In mid-water	None	A few unmet.
In sand (small portion)	Metd 28 Meting 0 Unmet. 1 (abnormal)	Metd 20 Meting 10 Unmet. 2

	(19. vi. 51) rinsed in sea water	(19. vi. 51) shaken on silk in sea water	(19. vi. 51) > 60-mesh sieved in sea water	(19. vi. 51) < 86-mesh sieved in sea water	(19. vi. 51) < 100-mesh sieved in sea water
Metd	0	0	0	0	0
Meting	8	13	2	0	0
Unmet.	17	17	12; *d* 1, poor cond. 5	9	5
Total	25	30	20	9	5

Dish B

	Salthouse Lake sand (St. II—19. vi. 51) rinsed in sea water	Salthouse Lake sand (St. II—19. vi. 51) washed and soaked in aquarium water for 5 days. Well rinsed in sea water	Salthouse Lake sand (St. II—19. vi. 51) washed with aquarium water and then kept in contact with mature *Ophelia* for 5 days. Well rinsed in sea water
Metd	7	10	0
Meting	17	9	3
Unmet.	10	6	4
Total	34	25	7

Dish C

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (18. vii. 50) rinsed in sea water
Metd	3	0
Meting	5	0
Unmet.	14	27
Total	22	27

Dish D

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51) washed in tap water, then in sea water	Bullhill Bank sand (19. vi. 51) soaked in sea water and absolute alcohol, then washed in sea water	Bullhill Bank sand (19. vi. 51) soaked in sea water neutral formol, then washed in sea water	Bullhill Bank sand (1947) washed, dried and subject to heating (900–1000° C.) in 1948
Metd	8	2	0	0	2
Meting	19	20	0	1	6
Unmet.	2	8	8	7	28, *d*. 9
Total	29	30	8	8	45

Dish E, strewn with lead-free ballotini > 86-mesh

On surface film	Many, mainly unmet.: a few early meting
In mid-water	Number swimming freely
In a portion of the ballotini	Metd 9 Meting 21 Unmet. 18 } ++

TABLE XIII. BULLHILL BANK SANDS

Attractive		*Neutral*		*Repellent*
Surface sand, fresh, untreated	Deeper sand, fresh, untreated		Washed in tap water, sterilized at about 100° C.	
	Fresh, washed for a few minutes in tap water		Soaked for several days in distilled water	
		After prolonged storage		
			Washed in tap water, dried at low heat	
Fresh, treated in tap water with activated charcoal	Washed in tap water, dried, treated with activated charcoal			
		Treated with hot conc. H_2SO_4		
Fresh, agitated on silk in sea water			Fresh sand treated with formalin or absolute alcohol	
		Heated 900–1000° C.		

TABLE XIV. SALTHOUSE LAKE SANDS

Attractive		*Neutral*		*Repellent*
		Surface sand from St. II, fresh and untreated		St. II sand washe in tap water, dried at low hea
		Surface sand from St. I, fresh and untreated		
				St. II sand washe in tap water, sterilized at abou 100° C.
		St. I sand, recombined, heated 900–1000° C.		
	Fresh St. II sand treated with activated charcoal in tap water			*St. I sand, dried and recombined. Extracted with alcohol, acetone or ether*
	St. II, sand washed in tap water, dried, treated with activated charcoal			
		St. I sand, washed and dried, treated with hot conc. H_2SO_4	*St. I sand, washed and dried, treated with* H_2O_2	

TABLE XV. MIXTURES AND VARIOUS

Attractive		*Neutral*		*Repellent*
Fresh Bullhill Bank sand + Bullhill Bank sand stored several years	Fresh Bullhill Bank sand + fresh Salthouse Lake (St. II) sand		Fresh Bullhill Bank sand + Salthouse Lake (St. II) sand washed and dried	
	Lead-free ballotini, mainly 60–86 mesh sizes		*Oolitic sand from the Great Salt Lake*	

TES ON THE BRITISH SPECIES OF *TRIDI-* *MNUM* (DIDEMNIDAE, ASCIDIACEA), WITH EPORT OF THE OCCURRENCE OF *T. NIVEUM* (GIARD) IN THE PLYMOUTH AREA

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(Text-fig. 1)

ill (1950) records two species of *Trididemnum* Della Valle (1881) as rring in British waters—*T. tenerum* (Verrill, 1871) and *T. alleni* Berrill 7). A third species, *T. niveum* (Giard, 1872), was reported from Millport ankin (1900), but it is impossible to determine whether his species was *iveum* or some other species of *Trididemnum* or *Didemnum*. Rankin's tifications are suspect, for he describes as common at Millport species of clinidae and Didemnidae which have not been seen in British waters (or ed, anywhere) before or since. *Trididemnum niveum* has not been reported e from Millport. The synonymy and distinctness of these three species confused. Hartmeyer (1924) stated that *T. niveum* is a synonym of *enerum*. This was denied by Harant & Vernières (1933), but without any ons of weight. Berrill (1947, 1950) claims that *T. alleni* is certainly nct from *T. tenerum*, but he allows the possibility that it may be nymous with *T. niveum*, which apparently he had never seen.

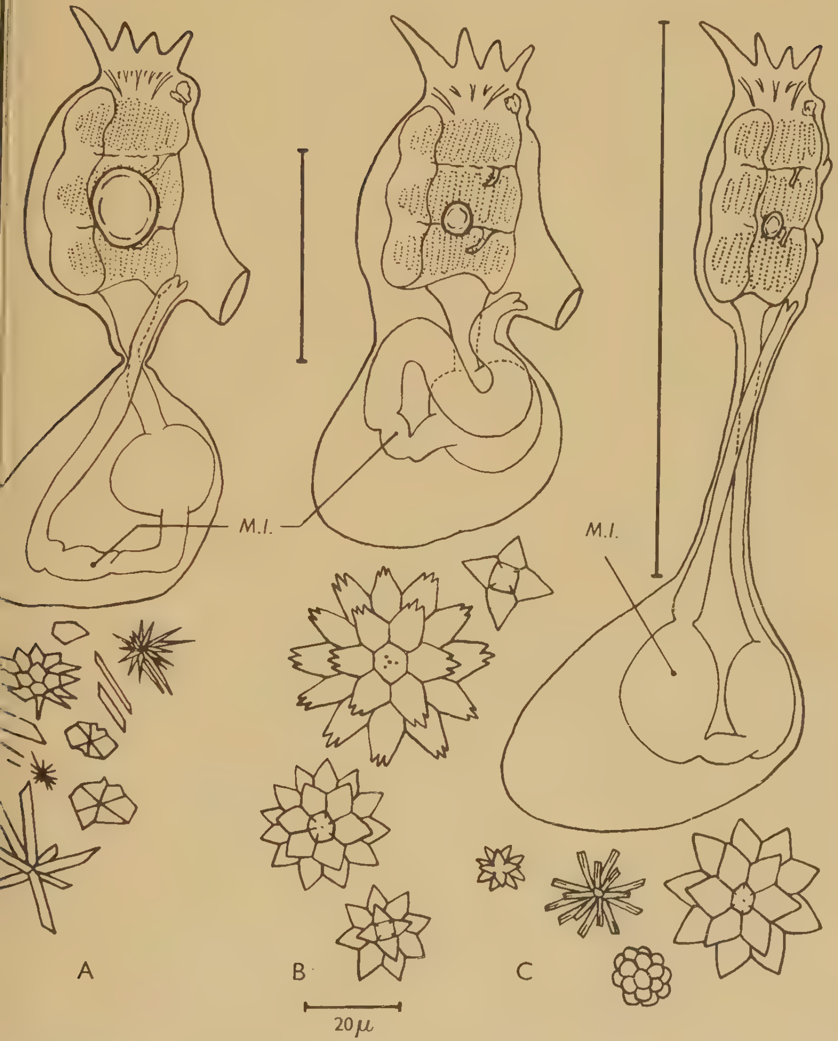
the Plymouth area I have frequently collected *T. tenerum* and *T. alleni*, I have in my possession colonies of *T. tenerum* from Naples and from land. Recently I collected a colony of a *Trididemnum* species from the tone (Salcombe, Devon); it was growing on the test of a specimen of *carpa rustica* (Linnaeus), a few centimetres below the low-water mark of ng tides. I ascribed this colony to *Trididemnum niveum*, and through the lness of Dr C. Levi I was able to obtain specimens of this species from the locality at Roscoff, including a portion of a colony from the type collection hat laboratory, determined by Pizon. I was thus able to check that my tification was correct. From the examination of numerous colonies of e three species of *Trididemnum* I have no hesitation in stating that they are hree distinct species.

. *tenerum* is found in waters from low tide down to about 200 m. It is monly found encrusting rocks and stones and on the holdfasts of algae. particularly abundant where the substratum is hard mud. *T. alleni* is rded from a little below low tide down to about 30 m., encrusting gorgonians, hydroids and algal holdfasts. It seems to require situations swept b strong currents or tides. *T. niveum* extends from a little below low-tide mar to about 30 m., and is especially abundant on the sargassids which inhabit th *Laminaria* zone. My specimens from Roscoff were all found growing o *Cystoseira.* Giard (1872) states that he never found it on stones or rocks. Lik many other ascidians, and especially didemnids, it seems to require an organi surface for settling.

Trididemnum tenerum forms large colonies of several centimetres in exten and 3 or 4 mm. thick. The colour varies through greenish, ochre, brown o grey, and the colonies are very rarely white. The zooids are usually visibl through the test; they are arranged in systems which may be obscured by th very abundance of the zooids. *T. alleni* forms much smaller colonies of th same thickness but rarely over 1 cm. in extent. The colonies are usuall brilliant white because of the abundance of the spicules, and for the sam reason the zooids are not visible. *T. niveum*, growing on *Cystoseira*, is limite in its extent in one direction, but in the other the colonies may extend to 60 o 70 mm.; the thickness is about 5 mm. The test is usually colourless, but th blue pigment of the abdomina combining with the white of the spicule produces the bluish white effect which led Giard to give it the specific nam 'niveum' or 'snowy'. In some colonies, however, the surface of the test i streaked with brown, and one group of colonies from Roscoff was in life dark yellow-ochre. The zooids in all my colonies are visible through th test.

The spicules of *Trididemnum tenerum* are rather irregular in form. Commonly elongate or needle-like crystals, or plates, are scattered in the test, but thes may be aggregated into irregular, or rarely regular, spherical groupings o 20–40 μ diameter; the points of these spherical spicules are usually needle-like and numerous. The spicules in most colonies are few in number an hardly obscure the transparency of the test. In *T. alleni* the spicules are ver closely packed, rendering the test completely opaque. They are spherical, o 8–25 μ in diameter and geometrical in form; looking into the sphere, in successive circles of points there are generally 1, 6 and 9 points, but rarely th formula may be 1, 4, 8 or, in the smallest, 4, 8. The rays are usually obtusel pointed but may be rounded or rod-like, even in the same colony. In *T* *niveum* the spicules are intermediate in abundance between those of the othe two species. They are spherical and geometrical in form with formulae o 1, 6, 12; 1, 4, 8; or 1, 4. I have not found any with the formula 4, 8. The are 20–40 μ in diameter with a few slightly smaller. The points are acute. I all three species they are most abundant in, or confined to, the upper layers o the test.

The thorax of the zooids of *T. tenerum* is at least as large as, or generall larger than, the abdomen and generally separated from it by a very narro constriction, which is, however, short. The thorax of *T. alleni* is alway



Drawings from the left side of zooids of A, *T. tenerum*, B, *T. niveum*, and C, *T. alleni*, gether with a group of spicules from each of the three species. Each group of spicules from a single colony. The bottom scale line refers to the degree of magnification of the icules. The zooids of *T. tenerum* and *T. niveum* are drawn to the same scale, but that *T. alleni* is enlarged more, so that it may be drawn with the same thorax size; in each se the scale line represents 0·5 mm. The entire genital system and the pyloric gland are nitted, so that the course of the gut—important for identification—may be followed. he buccal lobes and tentacles of the opposite side are omitted, so that one more than lf the total number of each are drawn. *M.I.* mid-intestine.

smaller than the abdomen and separated from it by a long neck which is about the same length as, or longer than, the thorax and rather narrow, though not strongly constricted. In *T. niveum* the thorax is of about the same size as the abdomen, sometimes a little larger and sometimes a little smaller; despite the drawing of Harant & Vernières (1933) it is uncommon to find a zooid with the thorax larger than the abdomen. The abdominal size is governed chiefly by the state of development of the testis. In *T. tenerum*, even when the testis is fully developed, the abdomen does not exceed the thorax. In *T. alleni* the gut-loop alone is as big as the thorax and the testis may be as big or bigger; the abdomen is thus always bigger than the thorax. In *T. niveum* the gut-loop is slightly smaller than the thorax, while the gut-loop plus a well-developed testis are together greater than the thorax; the usual condition seems to be one where there is a moderate-sized testis so that the abdomen is about the same size as the thorax. As stated by Giard (1872) the oesophageal region of *T. niveum* is very short and it is totally unconstricted. Indeed the thorax and abdomen in this species are not separated by the neck which is usual in didemnids. This lack of neck and the tortuous form of the rectum are the chief characters marking off *T. niveum* from other species of the genus.

Berrill (1947, 1950) reports that *T. alleni* has eight lobes to the branchial siphon, but neither Kott (1952) nor I have ever seen a zooid of this species with other than six lobes to the branchial siphon. The possession of eight lobes cannot be regarded as a diagnostic specific character. The other two species have six-lobed branchial siphons. The atrial aperture of *T. alleni* is a simple aperture opposite the second and part of the third rows of gill-slits. In the other two species it is produced into a definite funnel or tube which points posteriorly, and is situated behind the middle of the thorax, on a level with or posterior to the third row of gill-slits. In *T. tenerum* the opening is circular; in *T. niveum* it is transversely elongated to an oval form.

The lateral thoracic organ of *T. tenerum* is larger than the height of a single row of gill-slits; it is situated about midway along the side of the thorax. In *T. alleni* and *T. niveum* it is much smaller, though clearly visible. In these two species it lies a little behind the mid-point of the thorax. In neither species has it a raised rim such as is seen in *T. tenerum*.

A thoracic muscular retractor process may be developed in some zooids or colonies of all three species. All three commonly possess eight buccal tentacles, but large zooids of *T. tenerum* may have eight extra smaller tentacles making sixteen in all. *T. tenerum* and *T. niveum* possess nine to twelve stigmata in each half-row, with ten or eleven as the commonest number. *T. alleni* usually has seven or eight in each half-row.

The oesophagus of *T. tenerum* is more than half the length of the thorax, but less than its full length. That of *T. alleni* is longer than the thorax; that of *T. niveum* is less than half the length of the thorax. In all three it is straight and opens into a smooth globular stomach. The post-stomach of *T. tenerum* posteriorly and then turns ventrally. It is separated from the horizontal -intestine by an oblique constriction. The mid-intestine in its turn is rated from the ascending rectum by another constriction. The rectum is tly curved. In *T. alleni* the post-stomach is horizontal and opens after a t constriction into the ascending, globular mid-intestine which is as large r larger than the stomach. This passes without much break into the rectum ch runs practically straight up to the atrium. In *T. niveum* the post-nach is horizontal and is separated by an abrupt constriction from the small mid-intestine which runs almost without constriction into the um. This is curved into a tight S, quite unlike the condition usual in mnids.

errill (1947, 1950, p. 118) states that: 'Four or five obvious epidermal ullae with fairly long stalks and large terminals arise from the abdominal on' in *T. tenerum*, whereas in *T. alleni* they are hardly discernible. In *iveum*, Lahille (1890) saw five epidermal ampullae with voluminous inals. Giard (1872, pl. 22, fig. 1) illustrates *T. niveum* without epidermal ullae in contradistinction to '*Didemnum cereum*' (= *T. tenerum*) which he trates on the same plate (his fig. 3) with large ampullae. Berrill lays great s on this character, but I believe Van Name (1945, p. 99) is nearer the when he writes of *T. tenerum* that, 'at certain stages of growth clavate ular processes extending into the test from the middle region of the body found'. These are evidently the epidermal ampullae of Berrill. My own rvations indicate a great variability in all three species in the degree of lopment of these ampullae. While they are never very large in *T. alleni*, are sometimes clearly visible, and the other two species show all stages een highly developed ampullae and ampullae so small that they are only le in sectioned material.

he number of turns the vas deferens makes around the testis has some e as a specific character in the Didemninae, although the number is never gidly fixed as some authors seem to imply. In *T. tenerum* the number is lly given as twelve, and this is the commonest, but zooids may be found, in the one colony, with eleven or thirteen turns of the spire. In *T. alleni* is similar variation between seven and nine with eight turns of spire g most common. In *T. niveum* there are equal numbers of zooids with n and with eight turns while a very few have six or nine turns.

he larvae of the three species differ. The trunk of the larvae of *T. tenerum* *T. niveum* is about 0·45–0·5 mm. long; that of *T. alleni* is slightly smaller, g about 0·35 mm. long. The tail of the larva of *T. niveum* is longer than of *T. tenerum*, for it curves round the trunk even past the sensory vesicle, that of *T. tenerum* rarely passes the branchial siphon. The tail of *lleni* is of the same relative length as that of *T. tenerum*. The larva of *leni* has only two suckers, whereas those of the other two species possess suckers each.

The three species may be diagnosed as follows:

T. tenerum. Colonies up to 60 mm. across, usually with few spicules. Zooids always visible through test. Spicules chiefly irregular, 20–40 μ diameter. Abdomen never larger than thorax, and separated from it by a short but pronounced constriction. Atrial siphon prolonged to a tube, behind middle of thorax. Lateral thoracic organ near middle of thorax and larger than height of one row of stigmata. About ten or eleven stigmata per half-row. Oesophagus shorter than thorax. Post-stomach behind stomach; mid-intestine horizontal; rectum little curved. Vas deferens with about twelve turns around testis.

T. alleni. Colonies up to 10 mm. across, with abundant spicules. Zooids never visible through test. Spicules geometrical, spherical, about 8–25 μ diameter, with points arranged with formulae: 1, 6, 9; 1, 4, 8; or 4, 8. Abdomen always larger than thorax and separated from it by a narrow neck longer than the thorax. Atrial aperture a simple opening opposite the middle row of gill-slits. Lateral thoracic organ behind middle of thorax and smaller than the height of a single row of stigmata. About seven or eight stigmata per half-row. Oesophagus longer than thorax. Post-stomach ventral to stomach; mid-intestine large and globular, vertical; rectum little curved. Vas deferens with about eight turns around testis. Larva with only two suckers.

T. niveum. Colonies up to 60 mm. long, with sparse spicules. Zooids usually visible through test. Spicules geometrical, spherical, about 20–40 μ diameter, with formulae: 1, 6, 12; 1, 4, 8; or 1, 4. Abdomen larger or smaller than the thorax, and not separated from it by even the slightest constriction or neck. Atrial siphon prolonged into a tube behind middle of thorax. Lateral thoracic organ behind middle of thorax and smaller than the height of a single row of stigmata. About ten stigmata per half-row. Oesophagus shorter than half the length of the thorax. Post-stomach ventral to stomach; rectum curved into a sharp S, with final curve overlying stomach. Vas deferens with about seven or eight turns around testis.

SUMMARY

The presence of *Trididemnum niveum* (Giard) is reported from the Plymouth area. It is compared with the other two British species of *Trididemnum*, *T. tenerum* and *T. alleni*, with which it has in the past been somewhat confused, and all three are shown to be distinct species. A short diagnosis of the three species is given as an aid to identification.

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HE RESPIRATION OF SOME PLANKTONIC COPEPODS

II. THE EFFECT OF TEMPERATURE

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(Text-figs. 1, 2)

importance of the respiratory rate of copepods in considering the food
irements of zooplankton has already been stressed (Raymont & Gauld,
), and some of the factors affecting respiratory rates have been indicated.
perature has long been recognized as one of the most important factors
ting respiration, but apparently the determinations so far made on cope-
have been confined to one species, *Calanus finmarchicus* (cf. Marshall,
olls & Orr, 1935; Clarke & Bonnet, 1939). Since, especially in neritic
, other smaller copepods often predominate, it was felt that an investiga-
into the effects of temperature on some other species of copepod was
ssary.

MATERIALS AND METHODS

k was confined to three species of adult planktonic copepod, *Acartia*
i Giesbrecht,[1] *Centropages hamatus* (Lilljeborg) and *Temora longicornis*
ller). The copepods were obtained from tow-nettings taken in Southampton
r, between Hamble Spit Buoy and Baldhead Buoy, an area within 1–2 hr.
from the laboratory. The catch was filtered through coarse bolting silk
move ctenophores and large medusae, well diluted and kept in breffits
d in subdued light in the cold room of the laboratory at a temperature of
° C. Copepods were kept for at least 24 hr., sometimes for several days,
e cold room before use, in an attempt to avoid shock, and were fed with a
re of *Chlamydomonas* unless abundant food was present in the water.
tion of the copepods for the experiments was done as far as possible by
although a low-power microscope was used to a limited extent, and they
transferred from the stock breffits into crystallizing dishes of filtered
outh 'outside' sea water, before being put into the respirometers.

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[1] In a few experiments some *A. discaudata* were included.

Respiration measurements were made in Barcroft-Dixon constant pressure manometers as before (see Raymont & Gauld, 1951). Five ml. of sea water were used in each flask, which normally contained 45–55 copepods, and the flasks were shaken at a speed of 100 oscillations per minute, with an amplitude of 2·5 cm. Measurements were made either in the cold room or in the ordinary laboratory, depending on the temperature required, and in either case the flasks were immersed in a water-bath regulated to ± 0·2° C. of the selected temperature. Most of the experiments were conducted at temperatures ranging from 10 to 20° C., but some were carried out with *Centropages hamatus* at 6° C.

Each experiment ran for 3–4 hr. after an equilibration period of 15 min., and several readings were always made during the course of a run. Half-hourly readings were taken in the experiments at 20° C., but at the lower temperatures the manometers were read only hourly. The total change was then always converted to volume of dry gas at N.T.P.

After each experiment the copepods were fixed in formalin and the identifications checked under the binocular microscope. With a few exceptions the length of the cephalothorax of the fixed animals was also measured with a micrometer eyepiece (cf. Raymont & Gauld, 1951). Males and females were not separated in the experiments, but the number of each sex was always determined after fixation of the animals. Occasionally a few Stage V were accidentally included and sometimes one or two copepods of another species. The proportion of copepods other than adults of the selected species was always small, however. The lengths of all copepods were included in calculating the mean length for a particular run.

In two or three out of more than a hundred experiments, a nauplius or small zoea was inadvertently transferred to the experimental flask. Such animals were neglected in calculating the respiratory rates.

A very few runs showed no change in gas volume or very irregular changes and it is almost certain that leaks had occurred in the manometers. Such few runs have been disregarded.

RESULTS

Some 127 experiments were carried out altogether, and the respiratory rates, quoted as μl. oxygen consumed per copepod per hour, of *Acartia*, *Centropages* and *Temora* are given in Tables I, II and III respectively. In each table the experiments done at each temperature, viz. 20, 17, 13, 10° C., and for *Centropages* 6° C., are grouped together and the mean rate for each group is given. The mean length (cephalothorax) of the copepods used in each experiment is also indicated. Previous work (Raymont & Gauld, 1951) showed that the respiratory rate of several species of copepods was approximately proportional to the length squared, and since the mean length of the copepods in the present investigations varied considerably from one experiment to another, a corrected e for the respiratory rate was calculated in the following way. The overall n length of all animals used in all experiments was first determined for three species. These are: *Centropages*, 0·882 mm., *Acartia* 0·8585 mm.,

TABLE I. LIST OF EXPERIMENTS AND RESPIRATORY RATES OBTAINED FOR *ACARTIA CLAUSI*

Exact temp.	Exp. no.	No. of animals	Mean length (mm.)	Respiration rate (μl. O_2/cop./hr.)	Corrected respiration rate
20° C.:					
19·7	1	50	0·91	0·0935	0·083
19·7	2	50	0·92	0·122	0·107
19·7	3	51−1	0·92	0·121	0·106
19·7	4	50−2	0·92	0·0935	0·082
19·7	5	49	0·91	0·156	0·139
20·1	7	50	0·91	0·073	0·065
20·1	8	42−1	0·93	0·107	0·092
20·1	9	45	0·92	0·098	0·085
20·1	10	46−2	0·92	0·104	0·091
20·1	13	52−1	0·91	0·106	0·095
				Mean *0·107*	*0·0945*
17° C.:					
17·0	51	52	0·82	0·087	0·096
17·0	116	49−2	0·83	0·073	0·078
17·0	117	44−1	0·82	0·088	0·096
17·1	122	46	0·84	0·052	0·054
17·1	123	49−2	0·84	0·065	0·069
				Mean *0·073*	*0·079*
13° C.:					
13·1	36	48	0·85	0·075	0·077
13·1	37	53	0·87	0·072	0·071
13·1	39	49	0·82	0·0645	0·070
13·1	41	49	0·84	0·051	0·053
13·2	43	52	0·85	0·051	0·052
13·2	44	51	0·84	0·066	0·068
13·2	47	49−1	0·85	0·049	0·050
				Mean *0·061*	*0·062*
10° C.:					
10·7	16	41	0·85	0·062	0·064
10·7	17	46	0·79	0·069	0·082
10·4	21	41	0·85	0·132	—
10·4	22	45−2	0·79	0·087	0·104
10·4	23	41	0·83	0·086	0·093
10·4	24	50	0·81	0·045	0·051
10·4	25	51	0·82	0·0625	0·069
10·7	30	50	0·83	0·038	0·040
10·7	31	36−1	0·83	0·0535	0·057
10·7	32	50	0·83	0·043	0·047
10·4	98	53−1	0·90	0·042	0·039
				Mean *0·065*	*0·065*

e. In this and the following tables any copepods dying during an experimental run are by a figure subtracted from the total number used.

Temora 0·787 mm. The respiration rate for each experiment was then iplied by the square of this overall mean length and divided by the square e mean length for the particular experiment.

TABLE II. LIST OF EXPERIMENTS AND RESPIRATORY RATES OBTAINED FOR *CENTROPAGES HAMATUS*

Exact temp.	Exp. no.	No. of animals	Mean length (mm.)	Respiration rate (μl. O_2/cop./hr.)	Corrected respiration rate
20° C.:					
19·7	6	32	0·90	0·239	0·230
20·1	11	30 − 1	0·99	0·079	0·062
				Mean *0·159?*	*0·146?*
17° C.:					
17·0	48	58 − 3	0·92	0·076	0·071
17·0	50	53	1·06	0·126	0·087
17·4	57	50	—	0·0815	—
17·4	58	49 − 2	—	0·105	—
17·4	59	50	1·04	0·058	0·041
16·7	65	52 − 2	—	0·099	—
17·0	68	49	0·92	0·064	0·059
17·0	69	50	0·93	0·067	0·060
17·0	70	49 − 1	0·94	0·0595	0·053
17·0	118	51 − 1	0·83	0·108	0·122
17·0	119	46	0·83	0·104	0·117
17·0	120	53	0·84	0·085	0·092
17·0	121	47	0·83	0·109	0·121
				Mean *0·088*	*0·082*
13° C.:					
13·1	35	52	0·98	0·073	0·058
13·2	45	49	1·04	0·073	0·052
13·0	99	53	0·84	0·079	0·087
13·0	100	54	0·85	0·064	0·068
13·0	101	49	0·85	0·125	0·133
13·0	102	54 − 1	0·86	0·092	0·097
13·0	105	50	0·89	0·064	0·063
13·0	106	50 (?)	—	0·054	—
13·0	107	50	0·84	0·0785	0·085
13·0	108	48 − 2	0·86	0·115	0·119
13·0	109	51	0·86	0·106	0·111
				Mean *0·084*	*0·087*
10° C.:					
10·3	88	46	0·87	0·064	0·065
10·3	89	45 − 1	0·86	0·066	0·069
10·3	90	42 − 1	0·87	0·083	0·085
10·3	91	48	0·89	0·077	0·075
10·4	92	52	0·83	0·047	0·053
10·4	93	50	0·83	0·049	0·056
10·4	94	46	0·84	0·0535	0·059
10·4	95	52	0·84	0·071	0·077
10·4	97	51	0·81	0·058	0·069
10·4	115	68	0·84	0·040	0·044
				Mean *0·061*	*0·065*
6° C.:					
6·0	75	46	0·88	0·040	0·040
6·0	76	46	0·88	0·040	0·040
6·0	77	51	0·85	0·036	0·039
6·0	79	57	0·86	0·027	0·028
6·0	80	51	0·86	0·041	0·043
6·2	81	48	0·88	0·0255	0·025
6·2	82	49 − 1	0·87	0·030	0·030
6·2	83	50	0·88	0·0245	0·025
6·2	85	54	0·86	0·032	0·034
				Mean *0·033*	*0·034*

real difficulty encountered during the course of this work was that the
pods used had to be obtained by tow-netting and that the species most
monly found varied from one haul to another. Thus in the early part of
vork *Acartia* was very abundant with some *Centropages*, while towards the

TABLE III. LIST OF EXPERIMENTS AND RESPIRATORY RATES OBTAINED FOR *TEMORA LONGICORNIS*

Exact temp.	Exp. no.	No. of animals	Mean length (mm.)	Respiration rate (μl. O_2/cop./hr.)	Corrected respiration rate
20° C.:					
20·4	14	30	1·03	0·253	0·148
20·0	128	46	0·71	0·130	0·161
20·0	129	45 − 1	0·70	0·124	0·156
				Mean *0·169*	*0·155*
17° C.:					
17·0	49	50	0·95	0·143	0·098
17·4	53	52 − 2	0·89	0·089	0·070
17·4	54	51	0·91	0·085	0·064
17·4	55	57 − 1	—	0·123	—
17·4	56	48	—	0·120	—
16·7	61	50	—	0·115	—
16·7	62	50	—	0·101	—
16·7	63	49	—	0·112	—
16·7	64	55 − 1	—	0·149	—
17·0	72	41	0·75	0·164	0·182
17·0	73	46 − 1	0·78	0·116	0·119
17·0	74	40	0·81	0·120	0·114
				Mean *0·120*	*0·108*
13° C.:					
13·1	38	40 − 2	0·93	0·081	0·058
13·1	40	48	0·86	0·094	0·085
13·1	42	45 − 4	0·92	0·0945	0·069
13·0	103	47	0·73	0·1045	0·121
13·0	104	51	0·70	0·063	0·080
13·0	110	50	0·72	0·074	0·089
13·0	111	50	0·72	0·049	0·059
13·0	112	51	0·71	0·072	0·088
				Mean *0·079*	*0·081*
10° C.:					
10·3	87	53 (?)	—	0·084	—
10·4	113	59 − 2	0·71	0·051	0·062
10·4	114	62	0·71	0·059	0·073
10·0	124	48	0·72	0·071	0·085
10·0	125	44	0·72	0·099	0·119
10·0	126	47 − 1	0·73	0·063	0·074
10·0	127	47	0·72	0·062	0·075
				Mean *0·070*	*0·081*

very few *Acartia* were found and *Temora* was most abundant. Experi-
al runs were made with whatever material was most readily available, and
fore at some temperatures the number of experiments on one species may
been insufficient to give a reliable mean respiratory rate. Thus for *Centro-*
at 20° C. the mean (0·146 μl. O_2/cop./hr.) (Table II) must be accepted

with caution. With *Temora* (Table III) only three experiments were made 20° C. but here the mean of the corrected respiratory rates is 0·155 μl./cop./h with a S.E. of ±0·003 and therefore the result is significant.

Table IV. THE MEAN VALUES OF THE RESPIRATORY RATES

Species	Temp. (° C.)	No. of Exps.	Mean length	Mean respiration rate	Corrected mean respiration rate
Acartia	20	10	0·92	0·107 ±0·006	0·0945 ±0·006
	17	5	0·83	0·073 ±0·005	0·079 ±0·007
	13	7	0·85	0·061 ±0·006	0·062 ±0·004
	10	10	0·83	0·059 ±0·0055	0·065 ±0·007
Centropages	20	2	—	0·159?	0·146?
	17	13	0·91	0·088 ±0·006	—
		10*	—	0·086 ±0·0065	0·082 ±0·009
	13	11	—	0·084 ±0·007	—
		10*	0·89	0·087 ±0·006	0·087 ±0·009
	10	10	0·85	0·061 ±0·004	0·065 ±0·004
	6	9	0·89	0·033 ±0·002	0·034 ±0·002
Temora	20	3	0·81	0·169 ±0·027	0·155 ±0·003
	17	12	—	0·120 ±0·007	—
		6*	0·85	0·1195 ±0·011	0·108 ±0·016
	13	8	0·79	0·079 ±0·006	0·081 ±0·007
	10	7	0·72	0·070 ±0·006	0·081 ±0·007

* Excluding experiments for which there are no measurements.

Table IV shows the mean respiratory rates and the means of the correcte rates for each species at each of the temperatures used. The means of th corrected rates of course omit those experiments in which the copepods wer not measured and for which, in consequence, no corrected respiratory rate could be calculated. Clearly in most cases the difference between the means c the uncorrected and corrected respiratory rates is very small. The most marke exception is *Temora* at 20° C. (uncorrected mean 0·169 ± 0·027; correcte mean 0·155 ± 0·003). Table III shows that of the three experiments carried ou at this temperature with *Temora*, one (Exp. 14) was with copepods of large siz (average 1·03 mm.) and gave a high respiration rate (0·253 μl./cop./hr.) whil the two other experiments (Exps. 128 and 129) used small *Temora* (0·705 an 0·702 mm. respectively) and the respiratory rate was greatly lowered (0·13 and 0·124 μl./cop./hr.). The marked effect of size on respiration even for th same stage and species of copepod is therefore well demonstrated.

DISCUSSION

Temora *and* Acartia

The results given in Table IV show that in general an increase in respirator rate follows a rise of temperature for all three copepods, as with other animal For *Temora* and *Acartia* the rise from 13 to 20° C. is steady, though the cor clusions to be drawn from the experiments at 10 and 13° C. are less certai s Table IV shows that there is apparently a small rise in mean respiratory for *Temora* between 10 and 13° C.; but when the respiratory rates are ected for length the means are identical at these two temperatures. For *rtia*, the mean corrected rate at 10° C. is actually very slightly greater than ° C., though the difference is not significant. At these lower temperatures relatively smaller gas-volume change probably introduces larger errors in

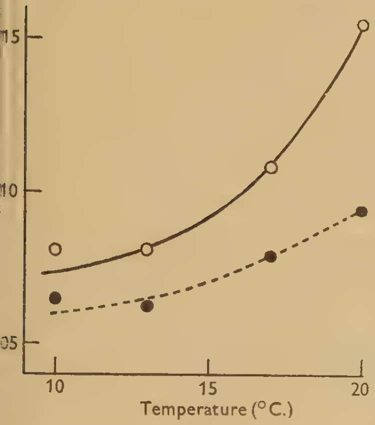

Fig. 1

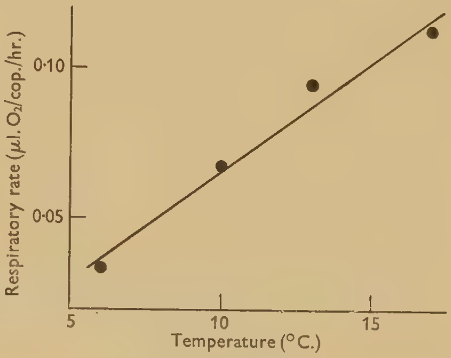

Fig. 2

1. The relation between respiratory rate and temperature. (Respiratory rate in μl.O_2 per opepod per hour). ○ and continuous line, *Temora longicornis*; ● and pecked line, *Acartia clausi*.

2. The relation between respiratory rate and temperature for the 2nd group of *Centropages amatus*.

xperiments, but as the curves given in Fig. 1 indicate, it seems clear that ise in respiratory rate with temperature for both species of copepods is er at lower temperatures and that the curves steepen from *c*. 15–20° C. has already been noted (cf. Zeuthen, 1947) for respiration/temperature ions for a wide variety of animals, and it agrees also with the results of shall *et al.* (1935) for the copepod *Calanus finmarchicus*.

g. 1 also indicates, however, that the respiration of the two species of pod is probably not affected to the same extent by the same rise in erature. Thus while for *Temora* the rate between 10 and 20° C. is almost led, for *Acartia* the rate increases only a little over 1½ times.

ese results may be compared with the temperature-respiration curve ned by Marshall *et al.* (1935) for *Calanus finmarchicus*. They worked at eratures down to 0° C., and they found that between this temperature 10° C. the respiratory rate for male and Stage V *Calanus* was approxi- y doubled. In females the increase was considerably less (0·28–0·40 μl. O_2/ hr.). Between 10 and 20° C., however, the curves for all *Calanus* steepen, and this agrees with the results here quoted for *Temora* and *Acartia*. Clarke & Bonnet (1939) found considerable variation in the respiratory rate of Stage V *Calanus*, but their results indicate a rise from 0·32 to 0·91 μl./cop./hr. for an increase in temperature from 5·5 to 16·8° C.

Centropages

In the experiments on *Centropages* there is a fairly steady increase from 6 to 13° C., but the rate at 17° C. (when corrected for length of animals) is slightly lower than at 13° C., though the difference is not significant. Closer examination of the results at 13 and 17° C. shows that at both temperatures the range in respiratory rate is unusually large: at 17° C. from 0·041 to 0·122 μl. O_2/cop./hr.; at 13° C., from 0·052 to 0·133 μl. O_2/cop./hr. (see Table II, corrected values). Of the experiments at 17° C. it is possible to separate Exps. 48–70 giving a range of 0·041–0·087 (mean 0·062) from the later experiments (Exps. 118–121) which show a range of 0·092–0·121 (mean 0·113). These two groups of experiments were carried out at rather different times: Exps. 48–70 from 8 to 13 June; Exps. 118–121 on 17 July, and it is probable that the two sets of experiments were carried out not only on different tow-nettings but also on different generations of *Centropages*. This idea receives some support from the length-measurements of the two groups of experimental animals; the mean of the first group (Exps. 48–70) is 0·965, that of the second group (Exps. 118–121), 0·833 mm.

Possibly the experiments at 13° C. can also be divided into two groups: Exps. 35 and 45 were carried out on 4 and 6 June with relatively large animals (mean length 1·014 mm.) and gave a mean respiratory rate of 0·055 μl. O_2/cop./hr., whereas Exps. 99–109 conducted on 21 and 22 June were concerned with smaller copepods, possibly again of a different generation (mean length 0·856 mm.) and gave a mean rate of 0·095 μl. O_2/cop./hr.

The first group of experiments at both 17 and 13° C. appears to have been performed on similar animals (mean lengths 0·965 and 1·014 mm.) at approximately the same time, 4–13 June. The rates for this generation at 17 and 13° C. are 0·062 and 0·055 respectively. The second group of experiments was carried out later on a smaller generation (0·833 and 0·856 mm. mean lengths), and gave rates at 17 and 13° C. of 0·113 and 0·095 respectively. If either group is considered, a rise in respiratory rate with temperature is clearly indicated and the extent of the rise is of the same order (13% for 1st group; 18% for the 2nd group), but it is obvious that the two groups must be considered separately. If the experiments carried out at the other temperatures can be assigned to either group it should be possible to get a real relation between respiratory rate and temperature.

The results at 20° C. cannot be seriously considered since only two experiments (which gave widely differing rates) were conducted at that temperature. At 10° C. (Table II) nine of the ten experiments were carried out on 19–20 June st (Exp. 115) about a week later. If only the first nine experiments be lered the mean of the corrected respiration rates is 0·068 (range –0·085). The mean length of the animals used in these nine experiments 48 mm., and the copepods are comparable to the second group at both ıd 13° C. Finally, the experiments at 6° C. were all conducted on June. The average length of the copepods is 0·867 mm. and the result ı respiration rate 0·034) should again be compared with those obtained the second groups of animals at 17 and 13° C.

BLE V. THE RESPIRATORY RATES OF TWO GROUPS OF *CENTROPAGES*

Temp. (° C.)	Group I (length 0·915 – 1·044; mean 0·977 mm.)	Group II (length 0·805 – 0·890; mean 0·854 mm.)
17	0·062	0·113
13	0·055 (?)	0·095
10	—	0·068
6	—	0·034

ble V shows these results for *Centropages* summarized. In Group 1 the are obviously too scanty to permit discussion, but in Group 2 a fairly rise in respiratory rate follows the rise in temperature from 6 to 17° C. experiments should be repeated on a more homogeneous brood of *opages*, but from Fig. 2, constructed from Group 2 data, it seems clear nce again van t' Hoff's law is not strictly followed (cf. *Temora*, *Acartia* lso *Calanus*).

Generation differences in Acartia *and* Temora

s perhaps necessary now to re-examine the data for *Temora* and *Acartia* whether different generations were used in the experiments with these ods, and whether variation between experiments might be correlated eneration differences. It must be emphasized that for all three species of ods, if respiratory rate can be correlated with generations, it cannot be ned on differences in length of the generations since all the rates have corrected to a standard mean length.

ome cases (e.g. *Acartia* at 17° C.) experiments were conducted several apart (e.g. Exp. 51 on 8 June: Exps. 116–123 on 17–19 July). But in l for *Acartia*, the mean lengths of the copepods used (though they show differences) and the corrected respiratory rates at any one temperature t clearly be separated into groups. In the experiments on *Temora* larger nces in the mean lengths and corrected respiratory rates were found vith *Acartia*, e.g. especially in the experiments at 13° C. But in general nge in respiratory rate found at each temperature is smaller than with *pages* and there is no obvious grouping of the experiments.

ile for *Temora*, and possibly even for *Acartia*, variations attributable to generation differences may distort the temperature/respiration curve, the evidence for such differences is not very strong and the curves given in Fig. 1 may be regarded therefore as showing a true relation between temperature and respiration for these copepods during June and July. It is still probable that different generations of these copepods at other seasons of the year might show very appreciable differences in respiratory rate, and a few preliminary experiments on *Temora* by one of us (J. E. G. R.) during the autumn give some support to this suggestion.

Differences in the respiratory rates of different generations might be suggested as an explanation of the puzzling results obtained previously with *Centropages typicus* (Raymont & Gauld, 1951). With this copepod it may be recalled that the experimental data fell clearly into two well-marked groups, one giving a mean respiratory rate of 0·29 μl. O_2/cop./hr., the second group the much lower mean of 0·18 μl./cop./hr. Differences such as time of keeping in the laboratory, feeding, and varying proportions of the sexes could not be correlated with these two groups. All the copepods used in these experiments were taken in one week (7–13 September 1949), so that the different respiration rates observed cannot be attributed to the use of different generations. But the copepods may have been taken from different populations since tow-nettings were taken both off Garroch Head, to the westward, and in Fairlie Channel, to the eastward of Cumbrae; unfortunately no records were kept of where the copepods used in any particular experiment were caught.

Sex and Respiration Rate

Marshall *et al.* (1935) have shown that male and female *Calanus finmarchicus* respire at different rates and are affected to different degrees by rising temperature. Since in the experiments here described male and female copepods were not separated, the suggestion might be made that variations in the respiratory rate, and in particular the variations seen for *Centropages* at 17 and 13° C., were due to different numbers of the sexes being used for experiments rather than different generations being employed.

The first group of experiments with *Centropages* at 17° C. (Exps. 48–70) together give a much lower proportion of females (56 % ♀♀) than the second group (Exps. 118–121) in which over 90 % were females. If, therefore, female *Centropages* respire more actively than males (though this is contrary to the findings of Marshall *et al.*, 1935, for *Calanus*), the higher respiratory rate in the second group of experiments at both temperatures might be attributed to the greater proportion of females. But reference to individual experiments at these temperatures shows that the suggestion is unsound. Thus at 17° C., in Exp. 48, with a great preponderance of males the respiratory rate was 0·071 μl. O_2/cop./hr., Exp. 69 with practically equal numbers was 0·060 and in Exp. 70, with three times as many females as males, it was 0·053, exactly the opposite of what would be expected.

hile, therefore, differences may exist between the respiration of male and le *Centropages hamatus* the marked variation between the two groups of riments at both 17 and 13° C. cannot be explained in this way. And d the possibility that instead we were really dealing with different rations of copepods is strengthened by the different proportion of the used in the experiments. For example, it has already been pointed out at 17° C. the second group of experiments (Exps. 118–121) has a much r proportion of females (more than 90%) than the earlier first group s. 48–70) with only 56% females. But since female *C. hamatus* are some- larger than males (cf. Sars, 1903; Marshall, 1949) the mean lengths ne copepods used in Exps. 118–121 would be expected to be greater in Exps. 48–70. In fact the copepods used in Exps. 118–121 were itely smaller than those used in earlier experiments, and it is there- even more probable that they belonged to a different generation. Marshall 9) has shown that the length of *Centropages* (Stage VI) decreases to a mum about July and August.

Length and Respiratory Rate

periments on a number of species and stages of copepods have suggested respiratory rate can be expressed as a function of the length (Raymont & d, 1951) in the equation

$$\log R = 2{\cdot}19 \log L - 0{\cdot}928,$$

e R = respiratory rate at 17° C., and L = length of cephalothorax. It is of est to see whether the present determinations on *Centropages*, *Acartia* *Temora* will also fit this suggested relationship. Table VI gives the lengths, corrected respiratory rates and the respiratory rates calculated from the tion quoted above.

TABLE VI. THE CALCULATED RESPIRATORY RATES AND THE ACTUAL RATES OBTAINED

Species	Mean length (mm.)	Corrected respiration rate	Calculated respiration rate
Centropages	0·882	0·082	0·090
Acartia	0·8585	0·079	0·085
Temora	0·787	0·108	0·070

ne agreement between the observed and calculated respiratory rates is nably good for *Centropages* and *Acartia*, but for *Temora* the calculated rate o) is considerably lower than the result obtained experimentally (0·108), therefore, in contrast to *Centropages* and *Acartia*, it would appear that the ested respiration/length relationship is only roughly applicable to *Temora*. obably one of the real factors determining the respiratory rate is surface and the length squared may be regarded as a measure of surface. A numerical ratio between the square of the length of a copepod and its surface area can be calculated if its cephalothorax is matched by a simple geometrical form.[1] While *Calanus*, *Acartia* and *Centropages* have elongate, oblong cephalothoraces which can be closely matched by a cylinder with a hemisphere at each end, that of *Temora* is relatively short and swollen anteriorly and can best be matched by two cones lying base to base, the longer one which forms the posterior part of the cephalothorax having its apex truncated. *Euchaeta* is more irregular in shape and the ratio quoted below was calculated by summation of a series of cylinders and parts of cones. From these forms the following ratios between the square of the length and surface area were calculated:

Species	L^2/S	Species	L^2/S
Calanus finmarchicus	1·03	*Centropages hamatus*	0·92
Euchaeta norvegica	1·13	*Acartia clausi*	1·19
Centropages typicus	0·96	*Temora longicornis*	0·72

It can be seen that in all the species except *Temora* the ratio is nearly 1, so that length squared is a fairly good measure of the surface area. That for *Temora* is much lower and its length must be multiplied by 1·2 before squaring to make it an equivalent measure of surface area. If the respiration rate of *Temora* is now calculated using 1·2 *L* instead of *L*, a value of 0·106 µl./cop./hr. is obtained which agrees very well with the experimental result.

General Conclusions

There remains the very difficult question as to how far results obtained from laboratory experiment may be applied to conditions in the sea. It is likely that the shaking of the apparatus and the concentration of some fifty small copepods in about 5 ml. of sea water for some hours may stimulate and excite the animals, so leading to a respiratory rate that is considerably higher than that under natural conditions (cf. Raymont & Gauld, 1951). But even if the rate quoted here for any given temperature is higher than in the sea, it is probable that a rise in respiration with temperature will follow in the sea just as in the laboratory. Temperatures of around 20° C. will certainly not be experienced in British waters, but a rise of 9–10° C. (say from 6 to 15° C.) between late winter and late summer is not unlikely in inshore surface waters and more striking temperature variations occur in a sea area like the Gulf of Maine. Riley (1946), for instance, quotes the mean temperature for the upper 30 m. as varying from 2·6° C. in March to 15·2° C. in September over Georges Bank (cf. also Riley, 1947, fig. 29).

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[1] The surface area so calculated is not the true surface area, since the surfaces of the urosome and the appendages have been ignored; in consequence the ratios given above have no absolute significance. But the calculated surface area probably bears a reasonably constant relationship to the real surface area so that the ratios quoted are valid for comparative purposes, as they are used here.

timates of the food required by planktonic copepods have been made by hall *et al.* (1935), Clarke & Bonnet (1939), Riley (1947) & Harvey (1950). e workers based their calculations on the respiratory rate of *Calanus rchicus* measured by Clarke & Bonnet (1939) or Marshall *et al.* (1935), ried to relate these requirements to the amount of available food in the Measurements of the respiration rate of other species (Raymont & Gauld, , and the present results) suggest that such estimates must be modified in ast two directions.

rst, it has been shown that the respiratory rate of copepods is approxi- ly proportional to the square of the length, probably because its magnitude termined by the surface area of the animals. Now all the estimates referred ove express the food requirements as a percentage of the weight of the al, giving values ranging from 2 to 8% at different temperatures. Since veight is roughly proportional to the cube of the length while respiratory and so metabolic food requirements, are proportional to the square of the h, the metabolic food requirements expressed as a percentage of the ht will be inversely proportional to the length. In consequence, estimates e food requirements of *Calanus* cannot be directly applied to other species mixed populations, and to estimate the food requirements of a population pepods one must know not only the total weight of the population but the size distribution of the copepods making up that population.

condly, in discussing seasonal variations in the respiratory food require- ts, these authors have assumed that temperature is the only factor ting the respiratory rate. The measurements of the respiratory rate of *ropages* described here apparently show that different generations of the species may have different respiratory rates (apart from differences ed to size differences of generations at the same temperature). If such ration differences should prove to be more wide-spread, then in addition n increase in food requirements during summer and a decrease in winter luced by seasonal changes in temperature, further changes, at present nown, in the food requirements of different generations will have to be n into account.

SUMMARY

respiratory rates of three species of planktonic copepods, *Acartia clausi*, *ropages hamatus* and *Temora longicornis*, were measured at four different peratures.

he relationship between respiratory rate and temperature was found to be lar to that previously found for *Calanus*, although the slope of the curves red in the different species.

he observations on *Centropages* at 13 and 17° C. can be divided into two ups and it is suggested that the differences are due to the use of copepods n two different generations.

The relationship between the respiratory rates and lengths of *Acartia* and *Centropages* agreed very well with that previously found for other species. That for *Temora* was rather different: the difference is probably due to the distinct difference in the shape of the body of *Temora* from those of the other species.

The application of these measurements to estimates of the food requirements of the copepods is discussed.

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URNAL VARIATIONS IN THE GRAZING OF PLANKTONIC COPEPODS

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(Text-figs. 1–3)

penny (1938) has shown that the percentage of copepods taken in the sea food in the gut tends to be higher at night than by day. Most planktonic als perform diurnal vertical migrations, of greater or less extent. penny's observations may well be a consequence of such migrations.

the other hand, during investigations into grazing rate of planktonic pods (Fuller, 1937; Gauld, 1951) some of the measurements have ested that there is a diurnal variation in the rate at which the copepods ct their food, even under laboratory conditions when migration is ssible, and that they filter the water more quickly at night than by day. variation in the percentage of copepods containing food may be caused ly by changes in the rate of feeding, this being controlled either by the nt of light in their environment or by some internal rhythm affecting behaviour.

investigation of diurnal variations in the feeding behaviour of *Calanus* *rchicus* (Gunn.) was undertaken to investigate (i) its behaviour in nature, ii) the possible existence of alternations of feeding and resting periods r laboratory conditions. This species was chosen because it is the nant copepod species in the Clyde sea area, is readily obtainable in nable numbers at all times of the year, and has already been the subject vestigation of feeding rate.

Field Observations

Methods

nus were obtained from 10-min. hauls of 50-cm. coarse tow-nets eshes to the inch) taken at 4-hourly intervals through 24 hr. Three nets fished at once, 25 fathoms apart on a trawl warp, to the end of which was hed a cable depressor (Barnes, 1951). Rigged in this way, the upper- net fished 2–3 m., the second 35–40 m. and the lowest *c.* 75–80 m. from urface. There was no means of closing the nets before hauling, but the ntage of the fishing time spent by the lower nets in the upper strata was l and unlikely to have interfered greatly with the results. The hauls were at a station just outside Tarbert, Loch Fyne. At this station deep water

(170 m.) was available only half a mile from the harbour where the research vessel could be tied up in harbour, and examination of the animals begun within 20 min. of their capture.

As Wimpenny (1937) has pointed out, the gut contents of *Calanus* are usually very easily observed, and in these investigations were estimated on the living animals as soon as possible after capture. Thirty to forty *Calanus* were transferred from the catch to a Petri dish and most of the water withdrawn so that the animals were compelled to lie on their sides and for the most part motionless. They were then examined under a low-power microscope and the amount of food in the gut recorded. This method of examination was adopted because it was found that in fixed catches, a variable and sometimes important fraction of the animals defaecated when formalin was added to the catch, and it was thought desirable to reduce as much as possible the interval between capture and examination. The number of animals examined varied from haul to haul, but an attempt was made to examine at least fifty, and, if time and numbers permitted, 100 of both Stage V and adult female *Calanus*. Occasionally Stage IV was sufficiently numerous for significant numbers to be counted in addition to the larger stages. After this had been done the catches were fixed with formalin and brought back to the laboratory. In some of the hauls the total numbers of *Calanus* or the numbers of one or other stage were too small for counts to be made quickly on board ship and supplementary counts of fixed material were made in the laboratory.

I am indebted to Skipper R. E. Souter and the crew of M.F.V. *Calanus* for their willing co-operation in taking the 24-hr. stations which comprise the field observations to be recorded here.

Results

Before anything is said about the gut contents of the catches, some remarks must be made about the vertical distribution of the animals. Since closing nets were not used the catches of the bottom net may have been made partly in the upper waters, but the differences in distribution to which attention are to be drawn are too marked to be attributed entirely to this cause, and in any case concern primarily the top net. The tables do not give the total numbers of animals caught (which were never estimated), but on any one occasion the catches of the top net were either of more or less the same size throughout the 24 hr. or else there was a marked difference between catches made in daylight and darkness so that the general picture of diurnal changes in distribution given Tables I and II is a true one.

It can be seen from the tables that only on seven of the thirteen occasions on which hauls were taken, were the catches of the top net distinctly greater in darkness than in daylight, while on 17 April and 11 September 1950, large catches of *Calanus* were taken at the surface throughout the 24 hr. On the four remaining occasions the catches varied rather irregularly and cannot be d on as clear evidence for or against vertical movements. It is remarkable clear evidence of migration was obtained only on two occasions in 1950 uly and 21 November), and even on 10 July the vertical movements were o clear cut as those of 1951, while in 1951 surface hauls in daylight were s blank except in April and May. The presence of adult and Stage V *us* in the surface water during daylight in spring and early summer has dy been recorded for the Clyde (Brook, 1886; Marshall & Orr, 1927; olls, 1933; Marshall, Nicholls and Orr, 1934), but it seems that a much percentage of the population than usual was present in the surface s in daylight on 10 April, and also on 11 September 1950, when exonally large catches were taken in daylight, although the day was not ually dull.

Females

e details of the observations on adult females are given in Table I, in the numbers examined from each haul and the percentage of these contained food is given. If a haul was taken and no *Calanus* seen this is as $(0)_0$; if a haul was not taken or was lost, it is indicated by a dash. The are given by the 24-hr. clock and are always Greenwich mean time.

e data of Table I are summarized in Table II, from which it can be seen he percentage of adult female *Calanus* found to contain food was cally identical in daylight and in darkness. These figures quite clearly h no evidence of diurnal rhythm in feeding.

the other hand, adult female *Calanus* were caught in all twenty-two e hauls taken in the dark, but only in twenty-seven out of the forty-eight ht hauls.[1] In consequence, more adult females were found containing by night (1275) than by day (1041), in spite of the fact that there were than twice as many daylight hauls as night hauls. These observations agree with those of Wimpenny (1938) that the feeding of *Calanus* takes mostly at night, but they also show that the reason for this is that by day *us* is usually absent from the surface water where their food is principally .

e numbers of female *Calanus* containing food in the deeper nets was always less than in the surface net, the lowest net of the three showing nallest percentage containing food. Of the adult females taken on all ons, 68 % of those in the middle net and 46·5 % of those in the lowest ntained food compared with 86 % in the surface net. The percentage of ls containing food varied considerably from one series of hauls to er—the mean for the bottom net from 16 % (9. i. 51) to 75 % (12. v. 51), f the mid net from 30 % (9. i. 51) to 86 % (28. ii. 50)—but in any given there is no obvious pattern in the occurrence of food in the guts unless there is clear evidence of vertical migration, when more of the copepods in the lower nets tended to have food in the gut at midnight and 4 o'clock than they had before midnight.

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[1] e hauls at 16.00 and 08.00 hr. were always taken in daylight, those at 20.00 and r. in daylight from May to August, and in darkness in the other months.

TABLE I. FEMALES: PERCENTAGE WITH FOOD

(The figures in suffix give the numbers examined.)

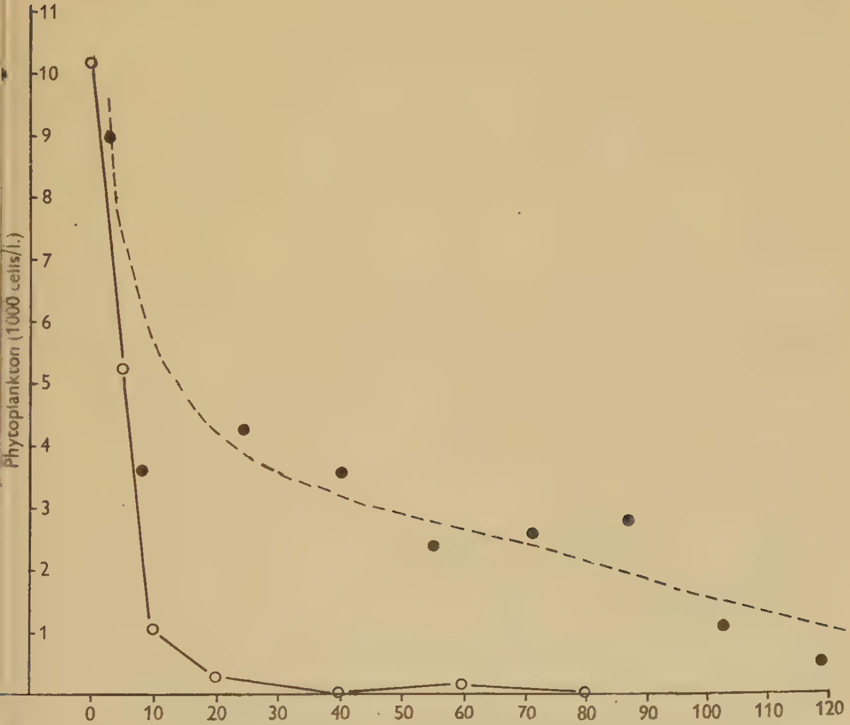
Time (hr.)...	12.00	16.00	20.00	24.00	04.00	08.00	12.00
28. ii. 50							
Top	83_{6}	89_{249}	94_{54}	75_{8}	98_{100}	90_{21}	69_{13}
Mid	100_{7}	91_{100}	75_{98}	88_{100}	89_{100}	87_{23}	83_{12}
Bottom	69_{88}	61_{100}	85_{102}	84_{74}	81_{100}	57_{100}	82_{100}
17. iv. 50							
Top	99_{100}	89_{100}	91_{100}	96_{100}	92_{100}	98_{67}	86_{100}
Mid	72_{100}	69_{100}	61_{100}	75_{100}	48_{50}	65_{79}	64_{98}
Bottom	52_{100}	24_{100}	45_{100}	28_{100}	56_{95}	36_{100}	51_{100}
29. v. 50							
Top	100_{5}	100_{2}	100_{4}	100_{4}	100_{1}	100_{3}	$(0)_{0}$
Mid	48_{50}	72_{50}	62_{50}	96_{50}	64_{50}	76_{50}	84_{50}
Bottom	34_{50}	54_{50}	64_{50}	64_{50}	56_{50}	64_{50}	44_{50}
10. vii. 50							
Top	29_{14}	$(0)_{0}$	67_{6}	83_{18}	$(0)_{0}$	50_{2}	0_{2}
Mid	0_{8}	40_{10}	32_{50}	91_{70}	43_{100}	48_{50}	$12{\cdot}5_{8}$
Bottom	39_{100}	40_{83}	69_{100}	84_{51}	79_{100}	26_{100}	20_{20}
11. ix. 50							
Top	78_{100}	91_{100}	78_{100}	88_{100}	73_{100}	74_{100}	92_{100}
Mid	57_{100}	64_{100}	68_{100}	77_{100}	82_{100}	71_{100}	70_{100}
Bottom	21_{100}	24_{100}	40_{100}	36_{100}	29_{100}	—	30_{100}
21. xi. 50			No females seen.				
9. i. 51							
Top	$(0)_{0}$	$(0)_{0}$	84_{19}	68_{19}	55_{9}	100_{1}	$(0)_{0}$
Mid	0_{3}	2_{100}	34_{83}	48_{101}	77_{22}	36_{11}	0_{19}
Bottom	0_{14}	0_{100}	15_{100}	12_{106}	16_{31}	18_{100}	0_{3}
14. ii. 51							
Top	$(0)_{0}$	—	99_{100}	99_{71}	99_{110}	$(0)_{0}$	$(0)_{0}$
Mid	$(0)_{0}$	—	85_{100}	94_{109}	83_{41}	44_{9}	13_{15}
Bottom	17_{17}	—	13_{100}	33_{100}	18_{100}	82_{82}	4_{52}
14. iii. 51							
Top	100_{3}	$(0)_{0}$	80_{5}	100_{5}	82_{43}	100_{1}	100_{3}
Mid	80_{8}	25_{4}	0_{5}	86_{35}	68_{71}	74_{23}	60_{5}
Bottom	63_{94}	47_{100}	36_{44}	50_{44}	33_{100}	0_{3}	40_{20}
5. iv. 51		Very few: top, 78_{18}; mid, 63_{11}; bottom, 72_{82}					
17. v. 51*							
Top	98_{62}	—	90_{41}	85_{100}	—	86_{100}	—
Mid	—	—	—	—	—	—	—
Bottom	76_{100}	—	72_{100}	87_{100}	—	67_{100}	—
4. 7. 51							
Top	$(0)_{0}$	$(0)_{0}$	$(0)_{0}$	100_{60}	$(0)_{0}$	$(0)_{0}$	$(0)_{0}$
Mid	17_{6}	0_{3}	20_{25}	96_{50}	82_{90}	$(0)_{0}$	$(0)_{0}$
Bottom	30_{50}	44_{50}	42_{50}	84_{50}	96_{50}	48_{50}	8_{50}
15. viii. 51							
Top	$(0)_{0}$	$(0)_{0}$	$(0)_{0}$	94_{50}	$(0)_{0}$	$(0)_{0}$	$(0)_{0}$
Mid	40_{35}	62_{50}	62_{50}	100_{50}	76_{60}	76_{50}	94_{50}
Bottom	16_{50}	44_{25}	18_{50}	78_{50}	84_{50}	4_{50}	38_{50}

* The series on 17 May 1951 was taken off L. Ranza and is incomplete: only two nets wer used and the times of the hauls were 13.00, 19.00, 22.00 and 07.30 hr.

TABLE II

	No. examined	No. with food	Percentage with food
Daylight	1190	1041	87·5
Darkness	1514	1275	84
Total	2704	2316	86

e decrease in the numbers of *Calanus* with food in the gut in deeper
and its dependence on the stratification of the food organisms was more
ly demonstrated on 11 September 1950 when a more detailed series of
was taken at six depths, and water samples taken at roughly corre-



Percentage of *Calanus* with food and phytoplankton concentration. Symbols: ●
d broken line, percentage of *Calanus* with food; ○ and full line, phytoplankton con-
ntration. Horizontal scale: depth in metres (0–120).

ding depths to show the distribution of the phytoplankton. The results
lustrated in Fig. 1 in which the phytoplankton (1000's of cells/litre) and
ercentage of *Calanus* with food are both graphed against depth. It can

be seen that the phytoplankton was concentrated in a narrow stratum about 10 m. deep, in which 90% of the *Calanus* were full; below this stratum the percentage fell from *c.* 40% at 10 m. to 5% at 100 m.

Adult Males

Male *Calanus* were not found in most of the hauls in sufficient numbers to obtain a clear picture of their behaviour. In addition they are relatively opaque and any food they may contain is less easy to see, so possibly small quantities of food, particularly in the anterior part of the gut, were overlooked. Of the 1555 adult males examined from all the catches 63% were in the lowest net, and 62% of them were empty. On only one occasion were more than twenty-five taken in a surface net (18. iv. 50, 08.00 hr.) when 38 were caught, of which 22 (58%) contained food; on the same occasion three samples from the mid nets contained over 50 males, of which 45, 73 and 44% contained food. Ten samples from the bottom net contained over 50 males and of these ten samples seven contained less than 40% males with food; in one (5. iv. 51, 24.00 hr.) 73% contained food. The amount of food was nearly always less than the amount contained by female and Stage V *Calanus*.

Stage V (Table III)

The behaviour of Stage V *Calanus* was very similar to that of the adult females (see Table III). Evidence of migration was found only in July and November in 1950, whereas in 1951 there was clear evidence of vertical migration in all the hauls except those in April and May. The percentage containing food is rather smaller; thus of 1541 Stage V *Calanus* examined from surface hauls made in daylight 1200 (78%) contained food, and 1181 (72·5%) out of 1630 examined from surface hauls at night contained food. But examination of Table III shows that the data can be subdivided: in most of the hauls the percentage containing food at the surface was over 70% and averaged 81%, a figure closely comparable with that for adult females. But on three occasions, 21 November 1950, 9 January and 15 August 1951, the percentages were much lower, averaging 50% containing food. Two of these occasions were in winter and the copepods may simply have been unable to obtain enough food, but this seems less likely in August, and since from August to January Stage V *Calanus* form an overwintering population it is possible that the small percentage containing food is due to a real change in behaviour.

On 11 September 1950, the percentages containing food were also abnormally low at night, averaging only 31% in agreement with the other observations in autumn. But on this occasion *Calanus* were present at the surface in considerable numbers during the day and of those taken by day at the surface 82% contained food, a percentage comparable to those found in spring and summer catches. The behaviour shown by the *Calanus* here is exactly the site of what would normally be expected because most of them were rently feeding *by day* and not at night.

TABLE III. STAGE V: PERCENTAGE WITH FOOD

(The figures in suffix give the numbers examined.)

(hr.)...	12.00	16.00	20.00	24.00	04.00	08.00	12.00
50		Very few: top, 60_{5}; mid, 38_{63}; bottom, 17_{30}					
. 50							
p	93_{100}	78_{100}	74_{100}	90_{100}	87_{100}	91_{80}	81_{100}
d	49_{100}	66_{100}	56_{89}	65_{100}	70_{100}	59_{100}	68_{100}
tom	41_{100}	25_{100}	26_{100}	49_{100}	48_{100}	33_{100}	34_{100}
50							
p	100_{31}	100_{31}	100_{10}	100_{17}	87_{23}	100_{26}	100_{21}
d	24_{50}	32_{50}	46_{50}	58_{50}	50_{50}	48_{50}	66_{50}
tom	26_{50}	36_{50}	52_{50}	32_{50}	30_{50}	48_{50}	30_{50}
i. 50							
p	53_{36}	100_{18}	93_{14}	98_{136}	0_{2}	81_{26}	89_{62}
d	21_{100}	25_{100}	31_{100}	46_{50}	28_{100}	35_{100}	18_{50}
tom	27_{100}	43_{97}	17_{100}	18_{50}	37_{100}	14_{100}	8_{50}
. 50							
p	99_{100}	92_{100}	20_{100}	36_{100}	38_{100}	49_{100}	88_{100}
d	74_{100}	83_{100}	18_{100}	16_{100}	17_{100}	26_{100}	73_{100}
tom	10_{100}	13_{100}	9_{100}	6_{150}	19_{100}	—	17_{100}
. 50							
p	$(0)_{0}$	$(0)_{0}$	18_{100}	47_{58}	52_{100}	0_{2}	$(0)_{0}$
d	10_{10}	4_{100}	10_{100}	13_{100}	14_{100}	0_{60}	0_{7}
tom	0_{100}	0_{100}	0_{100}	0_{100}	0_{100}	0_{100}	0_{100}
51							
p	$(0)_{0}$	$(0)_{0}$	89_{76}	58_{107}	51_{102}	100_{1}	$(0)_{0}$
d	0_{3}	2_{100}	36_{83}	48_{101}	77_{22}	36_{11}	0_{19}
tom	0_{14}	0_{100}	15_{100}	12_{106}	16_{31}	18_{100}	0_{3}
51		Very few: top, 100_{8}; mid, 0_{4}; bottom, 0_{30}					
. 51							
p	75_{8}	100_{1}	$87{\cdot}5_{32}$	87_{93}	95_{97}	100_{3}	84_{50}
d	10_{10}	79_{34}	79_{100}	97_{88}	91_{100}	97_{93}	89_{63}
tom	80_{90}	20_{100}	14_{100}	52_{100}	36_{88}	20_{15}	68_{89}
51							
p	60_{10}	78_{53}	$(0)_{2}$	87_{100}	95_{100}	63_{8}	100_{4}
d	98_{100}	96_{115}	96_{100}	95_{100}	96_{100}	80_{50}	75_{100}
tom	92_{100}	59_{100}	82_{100}	92_{100}	93_{100}	77_{100}	83_{100}
51 (see footnote, Table I)							
p	100_{16}	—	100_{37}	100_{12}	—	100_{59}	—
d	—	—	—	—	—	—	—
tom	71_{100}	—	64_{100}	51_{100}	—	44_{100}	—
51							
p	44_{9}	$(0)_{0}$	$(0)_{0}$	100_{50}	$(0)_{0}$	$(0)_{0}$	$(0)_{0}$
d	2_{50}	0_{17}	12_{50}	100_{50}	87_{100}	67_{6}	$(0)_{0}$
tom	8_{50}	8_{100}	22_{50}	36_{50}	43_{50}	13_{50}	3_{50}
i. 51							
p	$(0)_{0}$	$(0)_{0}$	$(0)_{0}$	38_{50}	$(0)_{0}$	$(0)_{0}$	$(0)_{0}$
d	32_{50}	20_{50}	42_{60}	92_{50}	6_{50}	18_{50}	20_{25}
tom	12_{50}	8_{50}	20_{50}	22_{50}	16_{50}	0_{50}	12_{50}

e percentage containing food in the lower nets, as with adult females, ess than at the surface, averaging 46% in the middle net and 29% in the t net.

Stage IV (Table IV)

On a few occasions, for instance on 29 May 1950, fair numbers of Stage IV were caught. When the numbers were sufficiently large to give significant counts, most of those in the surface water (averaging 91 % for all the samples in Table IV) and a smaller proportion of those from the deeper layers (38 % in the middle net and 23 % in the lowest net from Table IV) contained food, so that the behaviour appears to be closely similar to that of adult female and Stage V *Calanus*.

TABLE IV. STAGE IV: PERCENTAGE WITH FOOD

(The figures in suffix give the numbers examined.)

Time (hr.)...	12.00	16.00	20.00	24.00	04.00	08.00	12.00
29. v. 50							
Top	95_{102}	98_{112}	97_{110}	99_{104}	98_{109}	100_{100}	100_{100}
Mid	69_{62}	47_{90}	54_{50}	29_{50}	26_{50}	17_{50}	34_{50}
Bottom	12_{50}	13_{50}	28_{50}	13_{50}	16_{50}	25_{50}	28_{25}
11. vii. 50							
Top	91_{100}	98_{100}	84_{50}	93_{100}	100_{12}	98_{100}	76_{100}
Mid	22_{100}	18_{100}	19_{100}	26_{50}	21_{100}	23_{100}	20_{50}
Bottom	24_{100}	8_{100}	27_{100}	20_{50}	22_{100}	7_{100}	16_{50}
17. v. 51 (see footnote, Table I)							
Top	100_{14}	—	100_{34}	100_{9}	—	100_{24}	—
Mid	—	—	—	—	—	—	—
Bottom	75_{8}	—	100_{9}	9_{21}	—	63_{8}	—
4. vii. 51							
Top	(0)	(0)	(0)	100_{10}	(0)	(0)	(0)
Mid	11_{36}	12_{17}	12_{25}	90_{10}	91_{100}	15_{20}	(0)
Bottom	6_{50}	0_{30}	15_{20}	75_{20}	30_{23}	40_{20}	0_{9}
15. viii. 51							
Top	18_{50}	(0)	(0)	33_{15}	(0)	(0)	(0)
Mid	42_{7}	24_{25}	30_{10}	46_{50}	72_{40}	30_{50}	0_{9}
Bottom	12_{25}	16_{50}	16_{25}	6_{34}	6_{50}	15_{20}	21_{34}

Amount of Food

The amount of food in the gut varied from small quantities to a mass completely filling the mid-gut. An attempt was made to record the different amounts of food which the copepods contained. For example a *Calanus* with food throughout the length of its mid-gut was recorded as 'full', but a 'full' *Calanus* in April when diatoms were abundant obviously contained more food than a 'full' *Calanus* in winter, when phytoplankton was scarce; similarly, a 'full' adult female contained more food than a 'full' Stage V. These and other difficulties in maintaining a constant standard in a subjective estimate of this kind made the value of the estimates rather doubtful. The following general statements, however, are probably a fair summary of the observations.

The amount of food contained in the guts of the copepods with food was greatest in spring (April, May) when nearly all would be packed with food. During the summer the *Calanus* were not quite so well filled, but in winter

uantity was distinctly less, and small even in many animals at the surface.
amount of food in individuals captured in the lowest net was nearly always
than that found at the surface: for instance copepods with packed guts
rarely seen in the deepest catches. The *Calanus* in the middle net were
n intermediate condition; when food was abundant, most would contain
ge amount of food, but at other times the amount might be distinctly less
at the surface. As would be expected from their sizes, females contained
neral rather more than Stage V and these distinctly more than Stage IV;
s contained much less for their size than any of the other stages and in
ral contained distinctly less than Stage V.

nus finmarchicus *and* C. helgolandicus

es (1949) has drawn attention to the existence of two possible subspecies
finmarchicus and has suggested that they differ in temperature tolerance.
hall & Orr (1952) have recorded the presence of *C. helgolandicus* in the
e, occasionally in fair numbers, and have shown that the two forms differ
e time of spawning. It would be interesting to see whether its feeding
viour is also different. The catches were accordingly examined to see if
C. helgolandicus were present, but, except on one occasion, 11 September
, this form was absent altogether or made up an extremely small fraction
e catch. So far as could be seen from these small numbers the feeding
viour of the two forms was identical. It has already been seen that the
itions in September 1950 were unusual and the presence of *C. helgolandicus*
t explain the abnormality, but the numbers of *C. helgolandicus* do not vary
much from one haul to another, and since they did not rise above 32% of
catch, they are too few in number to account for the large changes in
ng behaviour observed. Further, the percentage of *C. helgolandicus*
aining food did not differ significantly from that of *C. finmarchicus*.

LABORATORY OBSERVATIONS

Methods

he laboratory the existence of a possible periodicity in grazing rate was
stigated, as in the field observations by direct examination of the gut
ents. The copepods were kept in a suspension of a food organism, usually
ecies of *Chlamydomonas* (cf. Gauld, 1951), and examined from time to
, usually at intervals of 4 hr., and the amount of food recorded.

Results

minary Investigations

efore the other experiments and any field observations were started, a
ber of experiments were made to see how long a copepod would take to
s gut when it began to feed and how long it would remain full after it had
ed feeding. The former were done by keeping copepods in sterile sea

water for some hours, usually overnight, to make sure they were empty. They were then put into suspensions of *Chlamydomonas* and examined at intervals of 10–15 min. to see how full they were. It proved very difficult to get any satisfactory observations. The transference of the copepods from a beaker to a watch-glass for examination disturbed them, and when they were returned to the beakers some time might elapse before they settled down, during which they darted about the beaker rapidly and normal feeding would not take place. The time taken to settle down varied quite erratically. However, in a moderately thick suspension of *Chlamydomonas*, comparable to those used in the experiments, *Calanus* can fill its gut in 10–15 min. at most and in thicker suspensions in a very few minutes indeed. It is unlikely that the natural phytoplankton on which the *Calanus* examined in the field were feeding would ever be as rich as that used in the experiments except possibly at the height of the spring maximum, but even in suspensions comparable in density to natural phytoplankton *Calanus* can fill its gut in less than 30 min.

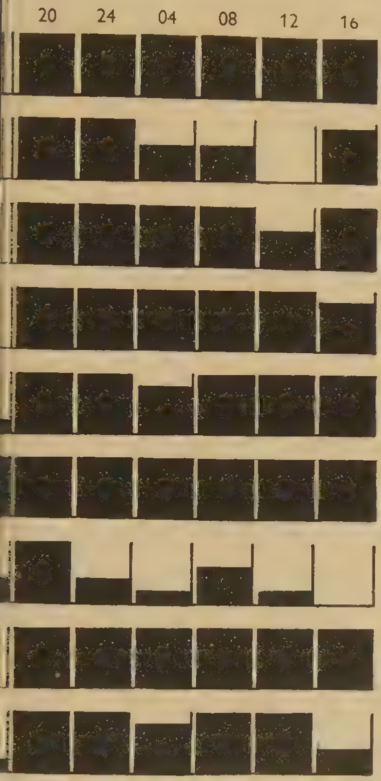
To find out how long food could remain in the gut the exact opposite procedure was followed. A number of *Calanus* were kept for some time in a fairly thick suspension of *Chlamydomonas* until they were full. They were then transferred into sterile sea water and examined as before at short intervals. Although a very small quantity of food matter may remain for several hours in the gut of a copepod which is swimming in sterile sea water, the bulk of the food will be digested and the undigestible residue passed out in about 1–2 hr.

Twenty-four-Hour Experiments

Observation of the amount of food in the gut gave no clear evidence of a consistent feeding rhythm, either for adult female or for Stage V *Calanus*. A typical series of observations is illustrated in Fig. 2. Each horizontal line illustrates the behaviour of an individual copepod and the vertical columns correspond to the hour of the observations. The quantity of food in the gut is given in an arbitrary scale—a column blacked to its full height means that the gut was quite full; three-quarters its height means that the anterior part of the gut was full but the posterior half was empty (usually because a faecal pellet had been passed out recently); half the height that the gut was only partly full, and one-quarter that only a small quantity of food was present. A white column means that the gut was empty.

It can be seen from the diagram that most of the adult females were feeding continuously throughout the 24 hr., the few drops in the columns were almost certainly due to the ejection of a faecal pellet just before examination. They were not, of course, perfectly consistent, as numbers 2 and 7 show. Stage V's (Fig. 3) seemed to be much less voracious and were much less predictable in their behaviour. Although feeding was not continuous and long gaps apparently intervened between meals, no consistent rhythm of feeding and resting can be deduced from these data. One point, however, emerges very plainly.

nus can spend a long time in a dense suspension of food without feeding me observations were carried on over several days, and although the



. Feeding of adult female *Calanus*. For explanation see text.

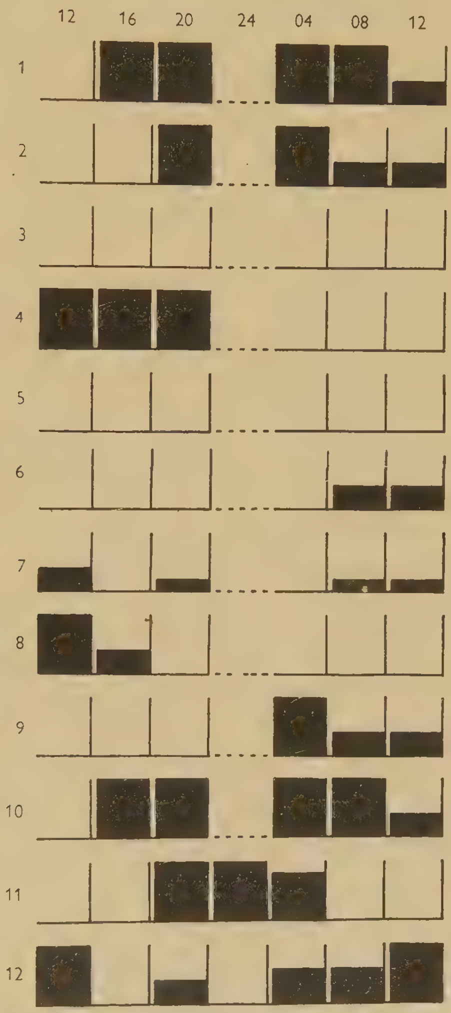


Fig. 3. Feeding of Stage V *Calanus*. For explanation see text. A broken line in 24 hr. column indicates that no observations were made at midnight.

pod appeared to be perfectly healthy and was seen, at least on some sions, swimming normally, no food was ever observed in the gut.

DISCUSSION

A copepod cannot be feeding, i.e. filtering water through its maxillae and ingesting the material accumulating on the maxillae, and yet have an empty gut for any length of time unless the water is nearly or absolutely barren. As the results of the preliminary experiments show, the gut fills up quite rapidly when a copepod is put into a suspension of food and, within an hour at most after starting to feed, it should contain sufficient food to make it clear that it is feeding. Similarly, when a full *Calanus* is put into barren water most of the gut contents are lost within 1–2 hr. of the cessation of feeding. It is plain, therefore, that if feeding and resting regularly alternated, this would be detected and quite clearly defined by estimation of amount of the gut contents at intervals through 24 hr.

Neither the field observations nor the laboratory experiments provide any evidence whatever of a diurnal rhythm of this kind. On the contrary, provided that sufficient food was available, the majority of the *Calanus* had full guts at all hours of the day and night and apparently were feeding continuously throughout 24 hr. Alternation of feeding and non-feeding periods, such as Wimpenny (1938) suggested, took place in the Clyde only when there was marked vertical migration of the copepods, into the surface water where food was abundant at night and down into deepet more barren water by day. When the phytoplankton is stratified in this way, diurnal vertical migration produces an apparent diurnal feeding rhythm, as was seen on many occasions in 1951—a pattern of feeding behaviour corresponding exactly with that observed by Wimpenny without the postulation of any innate rhythm in feeding activity.

There are two further points of interest arising out of these observations. On some occasions, such as 17 April 1950, no obvious vertical migration took place and *Calanus* were more or less evenly distributed at least as far down as the lowest net, 70–80 m. below the surface. As usual the numbers containing food in the catches of the lower nets were smaller than those in the upper net, and on this occasion only 39% of the *Calanus* (females and Stage V) in the lowest net contained any food and most of these (29% of the total) only a very small quantity. In the water in which they were caught these copepods were apparently unable to obtain enough food to maintain themselves. If the observations of 1950 were not all made on occasions when the behaviour of *Calanus* was abnormal, and obvious vertical migrations do not take place over long periods, it may yet be possible that although the population was not migrating up to, and down from, the rich surface layers all together as it does when the copepods display normal migratory behaviour, it was in a continuous state of flux, some *Calanus* migrating upwards to feed and others downwards out of the rich water at all times of the day. What stimuli would induce these movements in the individual *Calanus* is difficult to say unless they are hunger and repletion. In the absence of such movement, a considerable on of the population apparently remained in deep water where the cope- in time would starve.

condly, on most occasions in 1951 vertical migration was clearly marked he copepods spent the daylight hours in deep water, below the strata e food is abundant. In previous work (Gauld, 1951) I was able to show alanus was able to filter about 80 ml. in 24 hr., and the amount of food red by *Calanus* in 24 hr. has been estimated as 0·002–0·013 mg. (dry ht) (Marshall *et al.*, 1935). If the vertical migration is taking place, this nt of food must be collected not in 24 hr., but during the hours of darkness i.e. in 8–12 hr., depending on the season, and must be present not in l. but in 30–40 ml. of sea water.

SUMMARY

rds were made of the presence or absence of food in the guts of *Calanus rchicus* caught in three different depths, at intervals of 4 hr. through 24 hr.

was found that 80–100 % of the *Calanus* caught at the surface were full od at all hours of the day.

he number of *Calanus* containing food in deeper water was distinctly less. may be correlated with the abundance of its food close to the surface.

urnal vertical migrations took place on some occasions when samples taken, but not on all.

the absence of vertical migration *Calanus* was abundant at the surface eeding continuously at all hours of the 24 hr. Where vertical migration place feeding was mostly at the surface and was restricted to the hours of ness, i.e. in summer to a period distinctly less than 8 hr. round midnight.

aboratory observations confirm the absence of any feeding rhythm.

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NOTE ON THE ABSORPTION OF ORGANIC PHOSPHORUS COMPOUNDS BY *NITZSCHIA CLOSTERIUM* IN THE DARK

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ıs been observed by Chu (1946) that the marine diatom *Nitzschia clos-*
m, in bacteria-free culture, grows in the light with inositol hexaphosphate
hosphorus source, and with glycerophosphate.

hen this diatom is grown with inorganic phosphate as phosphorus source,
having used all the phosphate in the medium, further divisions take
, the cells becoming phosphorus-deficient. Then, on adding phosphate,
is rapidly absorbed by the deficient cells both in the dark and in light
chum, 1939).

periments have been made to find whether phosphorus-deficient cells
also increase their phosphorus content in the dark rapidly, if supplied
inositol hexaphosphate or with glycerophosphate.

ABSORPTION OF INOSITOL HEXAPHOSPHATE

acteria-free culture of *N. closterium*, obtained by subculturing after
essive growths in media containing penicillin and streptomycin (Spencer,
), was grown in artificial light. Two days after the phosphate in the
ium had been used by the diatom cells, equal volumes were transferred to
rifuge tubes. To each tube was added a solution containing 31 μg. P as
odium salt of inositol hexaphosphate together with inorganic phosphate
ent as impurity.

ne set of tubes was centrifuged at once and the liquid drained from the
sits of diatom cells, whose phosphorus contents were determined.

second set of tubes was stored for 3 hr. in darkness, before centrifuging
determining the phosphorus content of the cells.

third set of tubes was illuminated for 3 hr.

	Micrograms P in cells	
	Exp. I	Exp. II
ls separated by centrifuging immediately after addition of ganic phosphorus*	13·5	19
er 3 hr. storage in darkness†	35	42
er 3 hr. illumination	40	45

* The centrifugate contained 10 μg. phosphate-P.
† The centrifugate then contained 1·5 μg. phosphate-P.

Hence the increase in cellular P after 3 hr. darkness was twice as much as could have been supplied by the inorganic phosphate in solution, and indicates that 10–12 μ of organic P had been absorbed. The experiment does not indicate whether the organic phosphate was absorbed as such or converted by extracellular enzyme action to inorganic phosphate before absorption. However, a subsequent experiment using cells which were not phosphorus-deficient indicated very little, if any, conversion of inositol hexaphosphate to inorganic phosphate during 3 hr. storage in the dark.

ABSORPTION OF GLYCEROPHOSPHATE

A similar experiment was made in which (synthetic) glycerophosphate containing 45 μg was added to each tube containing equal volumes of phosphorus-deficient *Nitzschia* cells in suspension.

	Micrograms P in cells
Cells separated by centrifuging immediately after addition of glycerophosphate*	7·5
After 3 hr. storage in darkness	18
After 3 hr. illumination	20

* The centrifugate contained 3·3 μg. inorganic phosphate-P derived from impurity in the glycerophosphate.

This experiment indicates absorption of glycerophosphate as rapidly as of inositol hexaphosphate.

The sodium salt of inositol hexaphosphate was kindly supplied by Messrs Ciba Ltd.

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SYNTHESIS OF ORGANIC NITROGEN AND HLOROPHYLL BY *NITZSCHIA CLOSTERIUM*

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(Text-figs. 1–4)

n cells of *Chlorella* are grown in a medium containing a limiting quantity
itrate or ammonia as nitrogen source, photosynthesis and cell division
inue after all the available nitrogen in the medium has been abstracted
he growing plants. The cells become nitrogen-deficient. When kept in
ness with added nitrate these nitrogen-deficient cells synthesize a con-
able quantity of organic nitrogen (Ketchum, 1939) and use more oxygen
spiration than when kept in darkness without added nitrogen (Myers &
ner, 1948, p. 106). Growing in darkness with glucose as a carbon source,
use more oxygen when supplied with nitrate than when supplied with
onium, and even more carbon dioxide is evolved (Cramer & Myers, 1948,
), indicating a greater break-down of carbohydrate to supply the necessary
gy for synthesis of organic nitrogen compounds from nitrate than from
onium.

ese observations of nitrogen metabolism in *Chlorella* have suggested that
ms, and phytoplankton generally, may become nitrogen-deficient when
itrogen source is reduced to very low concentrations, such as occur in the
uring summer, and in consequence are able to absorb and build up organic
gen during the night (Harvey, 1945, p. 133). In order to obtain more
mation concerning nitrogen metabolism in phytoplankton the following
riments were made.

e marine diatom *Nitzschia closterium* var. *minutissima*, free from bacteria,
inoculated into autoclaved sea water of 26‰ salinity enriched with
phate, iron, manganese and a limiting quantity of nitrate or nitrite or
onia. The cultures were aerated and illuminated continuously by
escent lamps. Under these conditions, after a short lag period depending
the physiological state of the inoculum, exponential growth proceeds,
ng after the nitrogen source in solution has been absorbed by the cells
topping some 2 days later, when the cells attain a 'stationary state',
fully deficient in nitrogen.

hen these 'stationary' deficient cells were stored in darkness for periods
120 hr., there was no significant change in cell numbers, optical density
idity) of the culture, content of cellular nitrogen or of chlorophyll.
he other hand, when illumination was continued a slow decrease in
ophyll occurred.

In order to follow the rate at which organic nitrogen is synthesized by nitrogen-deficient *Nitzschia* cells in the dark when supplied with nitrate, ammonia or nitrite, or mixtures of these, four experiments have been made. Samples of the culture after adding a nitrogen source and storing in the dark were withdrawn at intervals and centrifuged, the deposit washed with sea water, and the cellular nitrogen determined by a micro-Kjeldahl method (Harvey, 1951). The results are shown in Table I and Figs. 1 and 2 (*Exps. 1–4*).

TABLE I. SYNTHESIS OF ORGANIC NITROGEN BY NITROGEN-DEFICIENT *NITZSCHIA* IN THE DARK

Nitrogen added at start of storage period (μg./ml.)	Increase as percentage of initial quantity of organic nitrogen present in 1 ml. of culture after storage in dark		
	Exp. 1 Cells in 1 ml. culture contained 0·75 μg. N and had been 3–4 days in stationary phase		
	After 24 hr.	After 48 hr.	After 120 hr.
4 μg. NH_4-N	230	240	—
4 μg. NO_3-N	125	140	140
4 μg. NO_2-N	Not significant	17	67*
4 μg. NO_3 + 4 μg. NO_2-N	125	125	125
	Exp. 2 Cells in 1 ml. culture contained 2·0 μg. N, 4 days in stationary phase		
	After 24 hr.	After 48 hr.	After 120 hr.
8 μg. NH_4-N	127	127	120
8 μg. NO_3-N	70	81	85
8 μg. NH_4-N + 8 μg. NO_3-N	130	129	—
	Exp. 3 Cells in 1 ml. contained 3·0 μg. N, 6 days in stationary phase		
	After 24 hr.	After 48 hr.	
8 μg. NH_4-H	147	143	—
8 μg. NO_3-N	73	73	—
4 μg. NO_2-N	10	13	—
4 μg. NO_3-N + 4 μg. NO_2-N	73	76	—
	Exp. 4 Cells in 1 ml. contained 2·0 μg N, 36 hr. in stationary phase		
	After 19 hr.	After 43 hr.	After 96 hr.
4 μg. NH_4-N	162	166	—
4 μg. NO_3-N	100	138	150
4 μg. NH_4-N + 4 μg. NO_3-N	145	167	—

* Analysis of the culture medium showed a loss in nitrite-N similar to the gain in organic-N by the diatom cells.

In order to determine which source was utilized when nitrogen-deficient *Nitzschia* was stored in darkness after the addition of both nitrate and ammonium, the following experiment was made.

Exp. 5. Nitzschia was grown with nitrate as nitrogen source. After 3 days in the stationary state, the culture was divided, additions made and the two suspensions stored 48 hr. in darkness. The ammonia in each (cells included) was then determined by microdiffusion (Conway, 1940).

Addition per ml. culture	Decrease in NH_4-N after 48 hr. in dark, per ml. culture (μg.)
4 μg. NH_4-N	3·92
4 μg. NH_4-H + 4 μg. NO_3-N	3·64

e experiment was repeated using a culture of N-deficient cells which had grown with ammonium as the limiting nitrogen source.

Addition per ml. of culture	Decrease in NH_4-N after 48 hr. in dark, per ml. of culture (μg.)
4 μg. NH_4-N	3·80
4 μg. NH_4-N + 4 μg. NO_3-N	3·64

e experiment shows preferential synthesis from ammonia in darkness. d been found previously (Harvey, 1940) that a mixed community of ne diatoms utilized ammonia in preference to nitrate when grown in light. *Exps. 1* and *3* there was only a 17 and a 13% increase in cellular nitrogen 48 hr. dark-storage with added nitrite. It seemed possible that this small ase was not due to synthesis from nitrite, as such, but from ammonia or te to which some of the added nitrite had been converted in the external um, by oxidation or by reduction due to an extracellular enzyme. In to explore this possibility the following experiment was made.

p. 6. Nitzschia was grown with nitrite as a limiting nitrogen source until ells were in the stationary phase. The cells in 1 ml. culture then contained nitrogen. The culture was divided, to one portion nitrite only was added, e other portion both nitrite and ammonium sulphate. After storage for . in darkness, the nitrite in both portions was estimated with the following t:

Addition per ml. of culture	Decrease in nitrite concentration after dark storage (%)
2 μg. NO_2-N only	25
2 μg. NO_2-N + 2 μg. NH_4-N	2·5

ce there was no significant change in concentration where ammonium lso added, it seems unlikely that nitrite is oxidized or reduced in the um before being slowly absorbed and utilized in darkness by the nitrogen-ent cells, unless nitrite-oxidizing or reducing bacteria had entered the re during the 48 hr. and had been inhibited by ammonia.

ese dark-storage experiments point to the following conclusions:

The presence of nitrate had little or no effect on organic nitrogen esis from ammonia in the dark, ammonia being utilized in preference to e (*Exp. 5*).

The presence of nitrite had little or no effect on the utilization of nitrate e dark (*Exps. 1* and *3*).

The quantity of organic nitrogen synthesized in the dark, by the same er of nitrogen-deficient diatom cells, from ammonia-N was greater than nitrate-N. This is presumably because more organic matter is lost by respiration when nitrate is utilized in order to provide the energy necessary for its reduction, and less remains for the synthesis of organic nitrogen (*Exps. 1–4*, Figs. 1 and 2).

(iv) Synthesis of organic nitrogen took place in the dark from either nitrate or ammonium by cells which could utilize nitrite only very slowly during a period of 48 hr. or more (*Exps. 1* and *3*, Fig. 1).

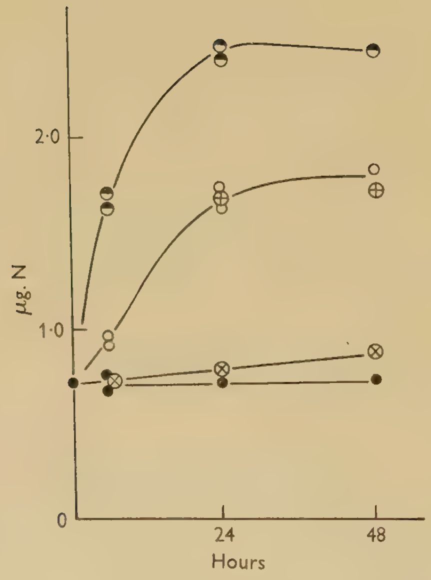


Fig. 1

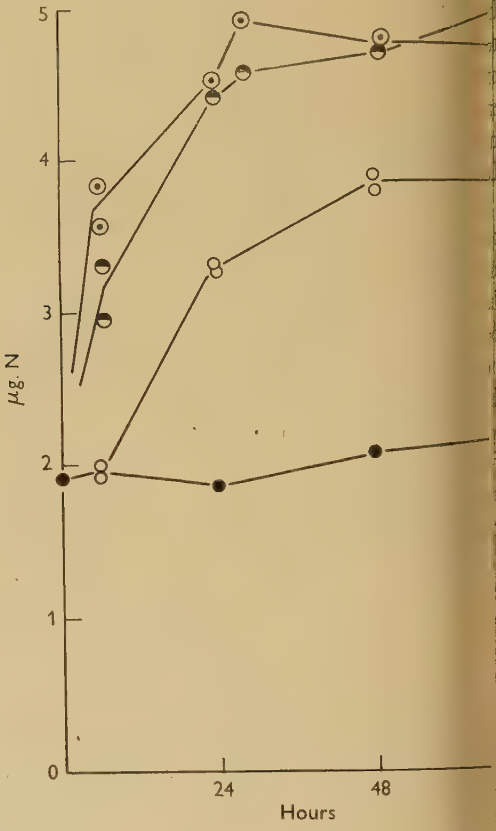


Fig. 2

Fig. 1. Micrograms organic nitrogen found in the cells contained in a cubic centimetre of nitrogen-deficient culture when stored in the dark after additions of nitrate, ammonia and nitrite (Exp. 1). ◓, 4μg. NH_4-N; ○, 4μg. NO_3-N; ⊕, 4μg. NO_3-N + 4μg. NO_2; ⊗, 4μg. NO_2-N; all added before storage in dark; ●, no addition.

Fig. 2. Micrograms of organic nitrogen found in the cells contained in a cubic centimetre of nitrogen-deficient culture when stored in the dark after additions of ammonia and nitrate. ◓, 8μg. NH_4-N; ⊙, 8μg. NH_4-N + 8μg. NO_3-N; ○, 8μg. NO_3-N; all added before storage in dark; ●, no addition.

A large number of observations have been made on the growth or division rate of this diatom at 17° C. at a light intensity which was such that a variation of ±30% had little effect. The cells were saturated with respect to light.

an initial lag, exponential growth proceeded as rapidly with nitrite or te as with ammonium as nitrogen source—the number of cells doubling 10½ hr. (C. P. Spencer, private communication).

hen deficient cells were stored in the dark with added ammonium, their gen content doubled or more than doubled in 10 hr. (Figs. 1 and 2). The riments provide no evidence that light enhances the rate of synthesis from onium nitrogen.

hen deficient cells were stored in the dark with added nitrate, the rate of esis was much less. In Exp. 1, where the most rapid dark-synthesis bserved, some 18 hr. were required for the cells to double their nitrogen ent. This suggests that light or some early product of photosynthesis may nce the rate of synthesis from nitrate.

hen deficient cells were stored in the dark with nitrite (*Exps. 1, 3* and *6*) little or no synthesis took place in 10 hr.

is shows that some transient product of photosynthesis, or light energy, nces the synthesis of organic nitrogen from nitrite very considerably.

hen deficient cells are supplied with nitrite and illuminated, growth in ers does not start until after a lag period of several hours. In order to tain the effect of light during this period, an experiment was made in deficient *Nitzschia* with added nitrite were stored both in darkness and light of a north window. The nitrite remaining was estimated.

ter 5 hr. in the dark there was a barely significant decrease in nitrite in ulture, whereas after 5 hr. illumination a third of the nitrite-N had peared.

y is nitrate absorbed and reduced to amino-nitrogen quickly in darkness nitrite is not reduced, although nitrite is the most probable first step in the of reactions by which nitrate nitrogen is converted to protein-nitrogen? sides nitrogen-deficient *Nitzschia* in darkness, neither the pulp of wheat (Burström, 1946) nor the crown-gall tissue of sunflower (Ricker & che, 1948) can utilize nitrite, whereas they can utilize nitrate.

stated by Burström, for wheat roots, this is inexplicable if free nitrite is uced as the first step in the reduction of nitrate. He suggests that nitrate t absorbed becoming firmly bound to cytoplasm, and then reduced to firmly bound to plasm colloid, no nitrite ions being set free.

hough this explanation can account for nitrite not being utilized by roots or sunflower tissue, it cannot wholly account for deficient chia not using nitrite in the dark yet using it in the light.

utilization of nitrite in the light it is necessary to postulate that some transitory product of photosynthesis either directly reduces the nitrite to oxime- or amino-nitrogen, or, alternatively, combines with the nitrite and allows reduction to proceed by enzyme action.

this postulate is correct, then it is possible that during reduction of te in the dark (when respiration is increased) a similar transitory product is set free which enables nitrite, formed as an intermediate, to be further reduced to amino-nitrogen. Such would account for the behaviour of *Nitzschia*, wheat root and sunflower gall tissue, and bring the observed phenomena into line.

These speculations assume that nitrite is the first intermediate when nitrate is utilized. There is no direct evidence for this. Bonner (1950, p. 225), reviewing nitrate reduction by plants, writes 'it is entirely possible that in some cases the reduction of nitrate may proceed through other reactions than the series of steps to be expected on the basis of inorganic chemistry'. Thus there is the possibility that in darkness *Nitzschia*, wheat roots and sunflower gall tissue do not reduce added nitrate via nitrite, and that nitrite is only utilized when it can combine or react with early products of photosynthesis.

Solubility of Organic Nitrogen Compounds Synthesized in Darkness

The following experiment shows that three-quarters of the dark synthesized nitrogen is soluble, compared with one-fifth of the total nitrogen in nitrogen-deficient cells.

Exp. 7. A culture of *Nitzschia* which had been stationary for 36 hr. was divided into four portions, to which additions were made as shown below. After 43 hr. in darkness, duplicate samples from each were centrifuged and the nitrogen in the cells determined. Also duplicate samples were centrifuged, resuspended in distilled water, heated for a few minutes in a boiling water-bath, again centrifuged and the nitrogen in the leached cells was determined.

	Micrograms nitrogen in cells per ml. of culture		
Addition per ml. of culture	Cells untreated	After leaching cells in hot water	Soluble N (by difference)
No addition	1·96, 1·97: mean 1·96	1·59, 1·63: mean 1·61	0·35
4 μg. NH_3-N	5·15, 5·30: mean 5·22	2·57, 2·91: mean 2·74	2·48
With 4 μg. NO_3-N	4·64, 4·70: mean 4·67	2·48, 1·98: mean 2·25	2·42
4 μg. NH_3-N and 4 μg. NO_3-N added	5·34, 5·27: mean 5·30	2·55, 2·53: mean 2·54	2·76

It is seen that the increase in organic nitrogen due to synthesis from ammonia or nitrate is largely in the form of water-soluble organic matter, while from the nitrogen-deficient control cells only 18 % dissolved.

	Increase in insoluble, and in water-soluble, nitrogen in cells per ml. of culture, due to synthesis in dark (μg.)	
Nitrogen source added	Insoluble N	Soluble N
Ammonia	1·13	2·13 or 65 %
Nitrate	0·64	2·07 or 76 %
Both	0·93	2·41 or 72 %

Chlorophyll Synthesis

uring the course of the foregoing experiments it was noticed that the gen-deficient cells became more heavily pigmented during storage in ness with added nitrate or ammonia, and also that during the growth of a ure the cells became less heavily pigmented before growth in numbers ed. These visual observations of the cells indicated that pigment synthesis limited by the supply of available nitrogen to the plant, and that synthesis s place in the dark if available nitrogen is added to the medium and hesized by the cells.

hese two aspects of pigment synthesis were examined in the following ner.

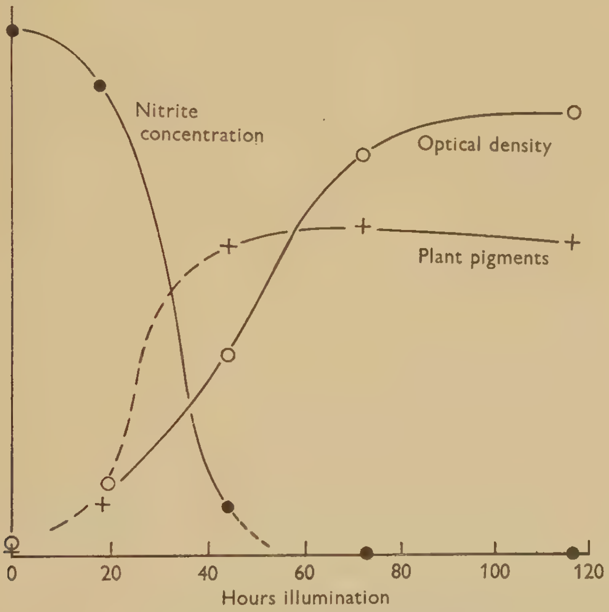


Diagram showing the decrease in nitrite in the water, increase in optical density and plant pigments in diatoms contained in unit volume of culture, of *Nitzschia* in 75 % a water enriched with 3 mg. phosphate-P, 0·1 mg. iron, 0·02 mg. manganese and 2 mg. itrate-N per litre (Exp. 8).

Exp. 8 a culture was grown with a limiting concentration of nitrite. At vals samples were removed whose nitrite concentration and optical ty were determined. The optical density provides a measure of the cell ers. In the method of determination used the relation was almost linear g the earlier growth, but towards the end of growth cell numbers ased slightly more quickly than the optical density increased. Samples also separated by centrifuging and the cells' pigments extracted with boiling methanol. The yellow-green extracts were matched against a series of colour standards (Harvey, 1934; Riley, 1941). Since the tint was in all extracts a very exact match, the proportion of yellow pigments to chlorophyll in each was approximately the same.

The determinations (Fig. 3) show that pigment synthesis ceased after some 45 hr. illumination at about the time when all the nitrite in the medium had been utilized by the cells, which continued growth, making about one division thereafter.

After 120 hr. illumination, samples of the culture were stored in darkness with and without the addition of nitrate. After 96 hr. dark storage the pigment content of the cells with added nitrate had increased by 70%, while that of the cells without added nitrate remained the same.

At the conclusion of *Exps. 3* and *4*, (p. 478) plant pigments were determined in the cultures.

Addition	Hours dark storage	Percentage increase after dark storage due to the addition: In N	In plant pigments
Exp. 2			
NH_4-N	120	120	75
NO_3-N	120	85	50
NO_3-N + Na_2S	120	60	25
Exp. 4			
NO_3-N	96	150	100

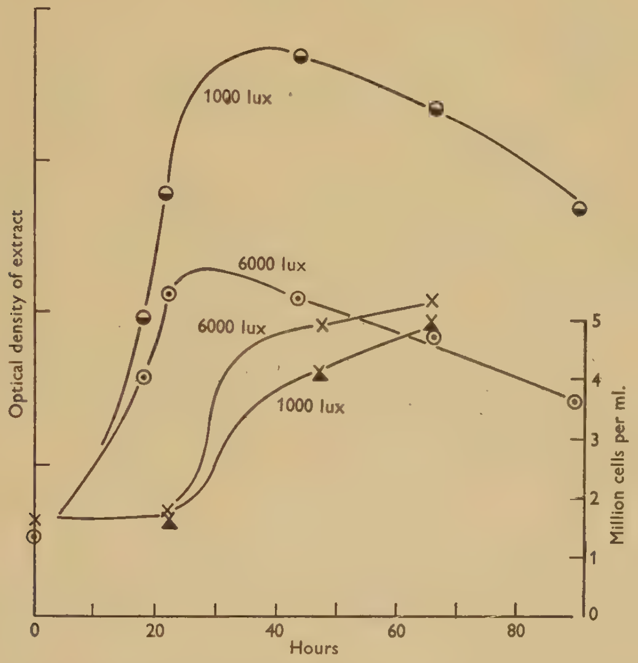
A well-marked relation between synthesis of nitrogen and of plant pigments in the dark is apparent.

Effect of Light Intensity on Chlorophyll Formation

It is noticeable that when this diatom is grown in media containing an ample supply of available nitrogen and phosphorus, the cells are more pigmented after growth in dim than in bright light. Visual observations with other marine species suggest, however, that it is not light alone which controls pigment synthesis during growth in culture.

Several experiments were made employing the following methods of estimation.

Light intensity was measured with a photometer calibrated in terms of mean noon daylight. The diatom cultures were illuminated with white light from fluorescent lamps. This light differs from daylight in being composed of a number of wave-length bands dispersed throughout the spectrum, although it is similar in respect to the mean intensities of each spectral colour. Since the sensitivity of the photometer varies throughout the spectrum, determination of the intensity of this white light only approximates to the values recorded as mean noon daylight, since this has a different composition in detail. However, in terms of overall intensity or light energy the recorded values should be a approximation. The greater light intensity used in the experiments oo lux) is similar to that in a north window on a moderately bright day. hlorophyll was measured either by determining the optical density of the w-green methyl alcohol extract in red light or with a spectro-photometer e wave-length for maximum absorbtion near 655 μ.



4. Diagram showing chlorophyll formation and destruction in a culture with limiting supply of available nitrogen, illuminated at 1000 and at 6000 lux (Exp. 9).

n *Exp. 9* sea water was diluted to 26‰ salinity and pasteurized, enriched h 3 μg./ml. phosphate-P, 2 μg. ammonium-N per ml., iron and manganese, was inseminated with *Nitzschia*, and divided into two Roux flasks. ne was illuminated at 1000 lux and the other at 6000 lux.

he course of chlorophyll synthesis and proliferation of the cells is shown ig. 4. I am indebted to Dr C. P. Spencer for haemocytometer counts of the numbers.

he inseminum was an old culture, and over 24 hr. elapsed before cell bers increased; meanwhile a considerable synthesis of chlorophyll had n place.

fter 45 hr. more than twice as much chlorophyll had been synthesized cell in those illuminated at 1000 lux as in those at 6000 lux.

Thereafter the chlorophyll in both cultures decreased, due to destruction by light, at a rate of half per cent per hour.

After 90 hr. illumination the cultures were stored in darkness for 6 days; no change in chlorophyll content occurred.

In order to find whether cells growing exponentially in moderate illumination in media rich in available nitrogen and phosphorus would increase their chlorophyll content when kept in the dark or transferred to dim illumination, the following experiment was made.

A culture growing at an average rate of one division in 10 hr. in 8000 lux was transferred to darkness. After 6 hr. dark-storage chlorophyll had increased by 10% and after 24 hr. by 14%.

Experiments have been made where the cultures were transferred from bright light (12,000 lux) to dim light (750 lux) and vice versa. They indicated that light intensity and supply of available nutrients are not the only factors affecting the quantity of chlorophyll per cell and affecting the ratio of yellow pigments to chlorophyll.

I am glad to acknowledge the help given to me during these experiments by Dr C. P. Spencer, who also supplied the bacteria-free cultures. To Dr W. R. G. Atkins, F.R.S., and Mr F. J. Warren, I am also indebted for calibration of the photometer.

SUMMARY

Experiments have been made with the marine diatom *Nitzschia closterium* grown in artificial light with different nitrogen sources.

After the nitrogen source in the medium was exhausted, photosynthesis and cell division continued, the cells becoming nitrogen- and chlorophyll-deficient.

On adding a nitrogen source to deficient cells and storing in darkness, synthesis of organic nitrogen, mostly water soluble, proceeded rapidly from ammonium, less rapidly from nitrate and very slowly from nitrite, with which it grows in light as rapidly as with nitrate or ammonium nitrogen.

Possible reasons why nitrate but not nitrite is reduced in the dark are discussed.

Chlorophyll and yellow pigments were synthesized in the dark by nitrogen-deficient cells in quantity related to the organic nitrogen synthesized.

Chlorophyll was synthesized in small amount by non-deficient cells which had been growing rapidly before transfer to darkness.

Cells contained less chlorophyll when grown in moderately bright than when grown in dim light.

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ASONAL VARIATIONS IN THE PHOSPHATE AND SILICATE CONTENT OF SEA WATER

PART VI. 1948 COMPARED WITH THE 1923–25 PERIOD

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(Text-figs. 1–4)

fifth paper of this series (1930) was the author's last, after which the work handed over to L. H. N. Cooper. It was resumed by the author in 1948 ompare the early years with the post-war, since the determinations had ed. Measurements of the extinction coefficient of the water were also med, and it was desirable to obtain the chemical data at precisely the e time.

he phosphate results are, as before, expressed as P_2O_5 in mg./m.^{3}, since crop calculations this old unit is convenient; to convert to mg. P, which ore rational, multiply by 0·437; to bring to milligram atoms multiply by 41. The analyses have not been corrected for salt error, since the early lts were not, and the factor for this correction has not as yet been agreed n.

he silicate results are given as mg./m.^{3} of SiO_2, but though the standard l in Plymouth was checked for me by Dr E. J. King in Canada and agreed his (King & Lucas, 1928), yet a different value had to be assigned to the a factor of the picric acid solution. All values have accordingly been tiplied by 1·44.

he phosphate results for E 1, surface, from 11 February 1948 to 1 February 9 are shown in Fig. 1 plotted against the dates. When one compares the sphate with the corresponding values for 1923 and 1925 it is obvious that winter concentrations are greater in the earlier years. But when considering ery yields it is not enough to compare the winter maxima, the summer imum must be taken into account and the difference fixes the minimum e of the plant production—minimum because some is used over again ing the year. The years 1925 and 1948 were alike in having a large spring ourst, so that most of the phosphate was used up by April or May. In 2 one sees the concentration of phosphate at each depth. The maximum sumption for 1923 was reached by 10 July, and was 29·6 mg./m.^{3}. (This calculation was rounded to 30 mg. and led to the value 1400 tons wet ght of phytoplankton per square kilometre down to 70 m.) Now taking

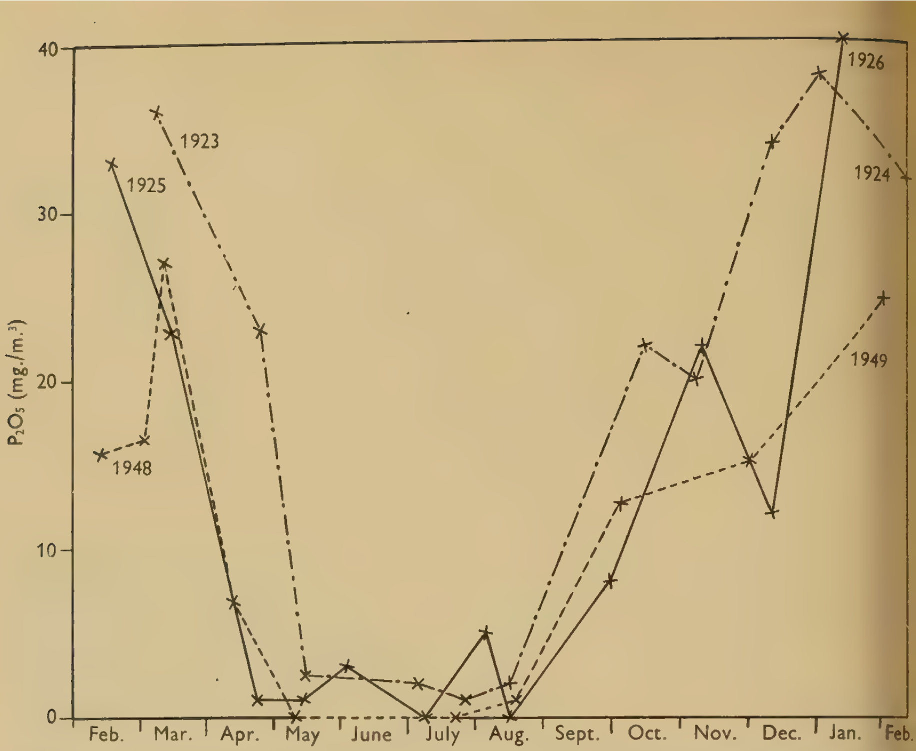


Fig. 1. Phosphate at Station E 1 (50° 02′ N., 04° 22′ W.), surface. Dash and dot, 1923; full line, 1925; broken line, 1948; each year continued into the early part of the next.

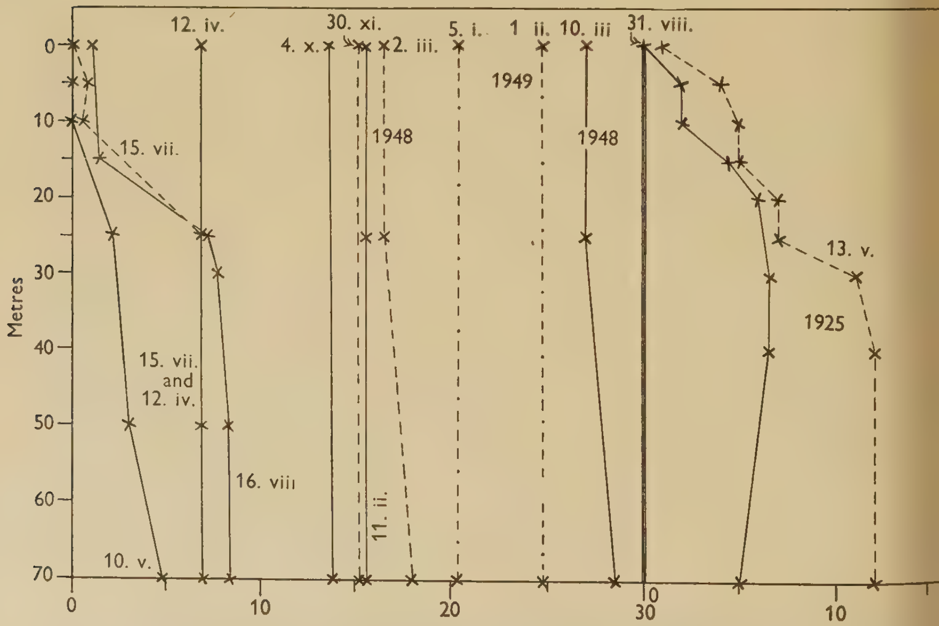
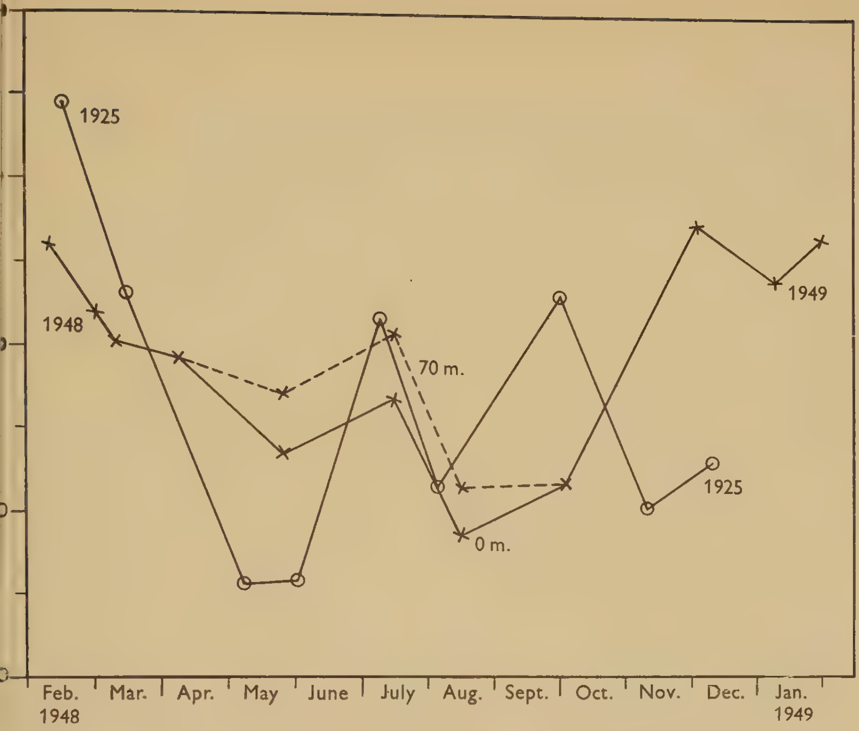
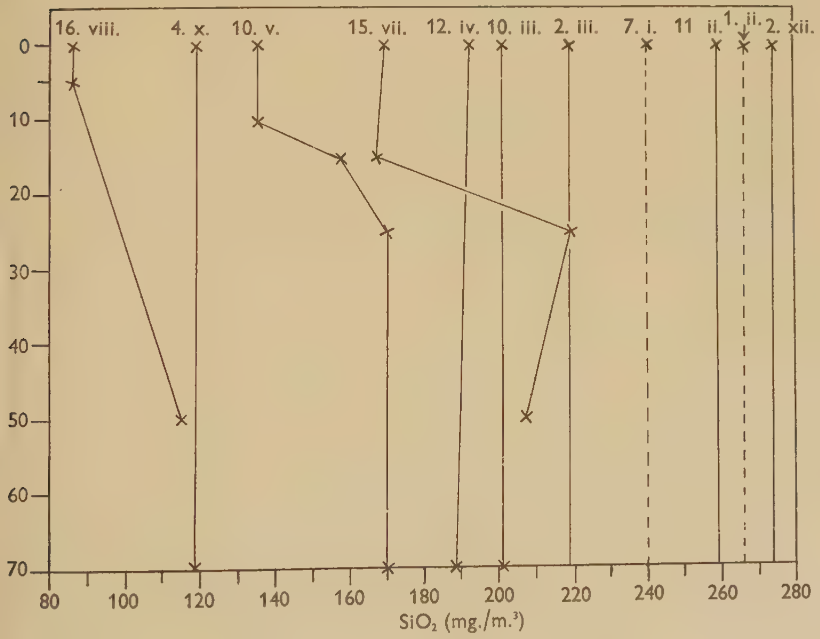


Fig. 2. Phosphate in water column at dates shown. Left-hand frame for 1948, some lines broken for clarity, and 1949, two only, dashes with dots. Right-hand frame for 1925.



. Silicate at Station E 1, surface. Full line 1948 to 1949, surface and bottom in winter; broken line 70 m. when distinguishable. Full line 1925, surface.



4. Silicate in water column at dates shown, 1948, with two dates in 1949, broken line.

29·6 mg. as 100 % we find that the yield for 1925 increased a little throughout the summer, and with a winter maximum just under 92 % of that for 1923, the production by 22 April, 13 May, 8 July and 31 August was respectively 80, 80, 89 and 94 % of that for 1923. As against this the winter maximum in March 1948 was 84 % of that for 1923, but because the utilization was more complete by 10 May it also was 84 % of the 1923 value after which regeneration of phosphate preponderated. Thus 1948, with less phosphate than 1925, produced more up till May, and then fell behind. The difference between production in 1923 and 1948 is only 16 %, whereas the decrease in winter phosphate maximum was 25 %. Thus it can hardly be said that there is a strict quantitative relation between the free phosphate supplies and net phosphate utilization. Fig. 3 compares the silica cycle for 1925 and 1948, surface. The values for 70 m. are also shown for 1948, dotted line. The winter maximum of silica for 1948 is almost 75 % of that for 1925 and very curiously the corresponding phosphate ratio is slightly over 75 %—the ratio may be taken as the same for each. Fig. 4 shows the silica variation with depth for 1948. Though the May results indicate a good development of phytoplankton, enrichment with silica is shown in July, and the year's silica minimum is in August, with 86 mg./m.^{3} at 0 and 5 m. and 115 below the thermocline. But the 1925 fall in silica occurred in April with surface 58 mg., which by 3 June had become uniform down to 70 m. It should be possible to get an idea as to whether diatoms or non-siliceous algae had been mainly responsible for a plankton outburst by a comparison of the relative decreases in phosphate and silicate.

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E SEASONAL VARIATION IN THE COPPER CONTENT OF SEA WATER

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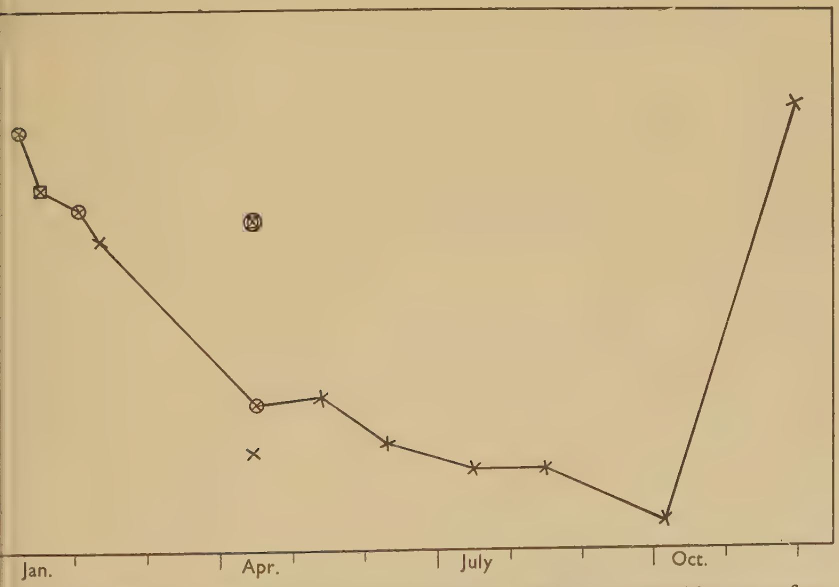
(Text-fig. 1)

evious papers (1932, 1933) the author showed that sea water taken from
nglish Channel contained about 10 mg./m.^{3} of copper, and obtained
ment between electro-deposition with subsequent colorimetric analysis
olorimetric analysis carried out on sea water concentrated to one-fifth of
lume. For extraction of the copper complex, carbon tetrachloride was
to be the most convenient solvent. The changes throughout the year
however, only recently been determined. It is of interest to see that
from Station E1, surface, taken 23 March 1931, gave 10 mg./m.^{3} by
colorimetry and 9·7 with electro-deposition, whereas the extraction
od curves for 1948 and 1949, read off for the same date, showed
ctively 9·4 and 11·4 mg./m.^{3}.



The abscissae show months, the ordinates copper in milligrams per cubic metre of sea
ter. Results for surface water at International Hydrographic Station E1 (50° 02′ N.,
° 22′ W.) are denoted by crosses for 1948, crosses in circle for 1949—with one in a circle
d a square also for 50 m., and by a cross in a square for 1950, one only, in January.

Early attempts to examine deep water were given up on account of the solubility of the metal bottle, but in 1949 a plastic-lined bottle was temporarily satisfactory and, on 13 April, 50 m. gave 18·3 mg. Cu as against 8·1 for the surface, sampled with a wooden bucket. Copper is thus a minor constituent which is taken up by the phytoplankton. The results for the surface water during 1948 and 1949, together with one observation for January 1950, have been combined in a single curve, as shown in Fig. 1, in which, after an autumn minimum of 1·5 mg./m^3, a winter maximum 24·8 is reached. The colour given by the high values was a rather browner tint than expected, so presence of iron was suspected. It had, however, been shown in the 1932 paper that it required 1·32 mg./l. ferric iron to equal in depth of colour 0·01 mg./l. copper. Moreover, Cooper (1935, 1937) showed that no more than 2·2 mg./l. ferrous iron could remain in solution in sea water and that the amount as ferric in solution was far beyond colorimetric detection. Ferrous iron could thus have introduced no serious error in the copper analyses. Miscellaneous analyses by Meyer (1938) showed 6–15·5 mg./m.^{3} copper in the Baltic, 9–26 mg./m.^{3} in Kiel harbour, 10 mg./m.^{3} in the North Sea, 8–11 mg./m.^{3} in the Bay of Biscay, with 12 mg./m.^{3} in the Canary Stream.

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SEASONAL CHANGES IN THE PHYTOPLANKTON DURING THE YEAR 1951–52 AS INDICATED BY SPECTROPHOTOMETRIC CHLOROPHYLL ESTIMATIONS

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(With Text-figs. 1–8)

previous paper (Atkins & Parke, 1951) the phytoplankton was studied by
ollodion filtration of 20 l. samples, the extraction of the filter disk with
aqueous acetone and the comparison of the extracts in a Kober colori-
r against each other and a commercial chlorophyll preparation. The
erence of pigments other than chlorophyll was eliminated or much
ed by the use of a Schott RG 1 filter, which we subsequently found had
ously been thus used by Rohde (1948). There was, however, frequently
ay in the filtration of our large samples and an increase in accuracy was
able.

r the present work we were fortunate in having a Unicam spectrophoto-
r with 4·0 cm. rectangular glass vessel for which 10 ml. of extract
ed. This was prepared usually the day after the water had been collected,
or 2 l. of water were sufficient, according to season. Unfortunately, the
vessels got broken, so for a time 1·0 cm. vessels were used and the
ts obtained for March and April were necessarily less accurate. The
urements of percentage transmission, as adjusted against a blank with
nt, showed a strong absorption in the red and an even stronger one in the
violet. Results were, however, based on the minimum transmission in
ed, since the shorter wave-absorption band was frequently masked by
eral absorption occasioned by carotins and xanthophylls. The accuracy
spectrophotometer was checked at 6563 and 4861 Å. and found to be
, using the hydrogen lamp.

The Spectral Absorption Curves

g. 1 the maximum absorption percentage of the red absorption band at
μ is plotted against the concentration of the commercial chlorophyll in
. The relation is strictly rectilinear up to 4·0 mg./l., and by 8·0 has fallen
8 % against 35·8 for rectilinearity. It would appear from the position of

the band maximum that the preparation contained both chlorophyll *a* and *b*. Fig. 2 shows the absorption curves of two Chlorophyceae, in pure culture, for which we are indebted to Dr M. Parke. That for *Chlorella* I (by F. Gross) shows the chlorophyll absorption bands with minimum transmissions at 655 and 420 mμ, but for *Chlamydomonas* III (by Mrs Foyn), a solution of greater

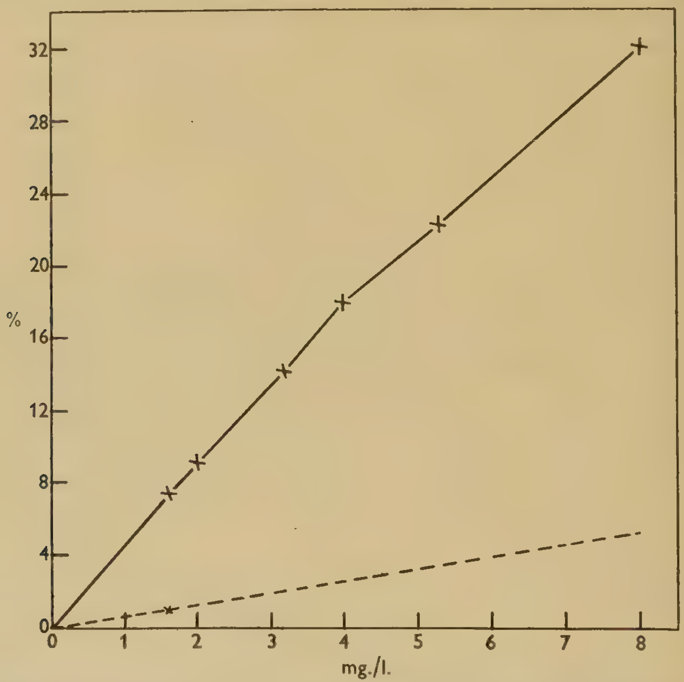


Fig. 1. Percentage absorption at 635 mμ (the position of minimum transmission in the red) for an 80 % aqueous acetone solution of a dry commercial chlorophyll the concentrations of which are given on the abscissae in mg./l., equivalent to 10 mg./m.^{3} when using 10 ml. plankton extract from 1 l. of sea water.

density also has a minimum at 655 mμ but shows complete absorption before the 420 mμ band has been reached. The same is shown by a strong solution, 320 mg./l. of the commercial chlorophyll, which gave maximum absorption at 640 mμ.

In Fig. 3 is shown an extract of *Hemiselmis rufescens*, with sharp absorption maxima at 655 and 435 mμ, also the commercial chlorophyll, 16 mg./l., showing maxima at 635 and 410 mμ. The characteristic buff colour of the alga is due to the presence of a water-soluble crimson, which remains on the collodion or paper and is not dissolved by the aqueous acetone. The wider bands exhibited by the chlorophyll solution are probably due to admixture of the two forms.

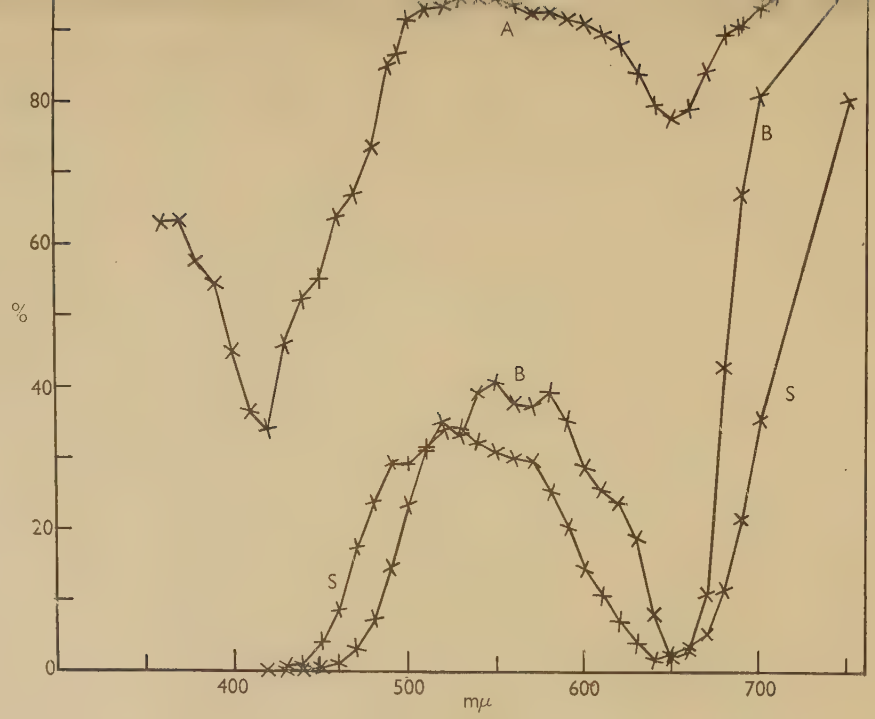


Fig. 2. Percentage transmissions from: S, a stock solution of a dry commercial chlorophyll in acetone, 90 %, with water; A, an 80 % acetone extract of a culture of *Chlorella* I; and B, the same of *Chlamydomonas* III. The abscissae in this and the three following figures show wave-lengths in millimicrons.

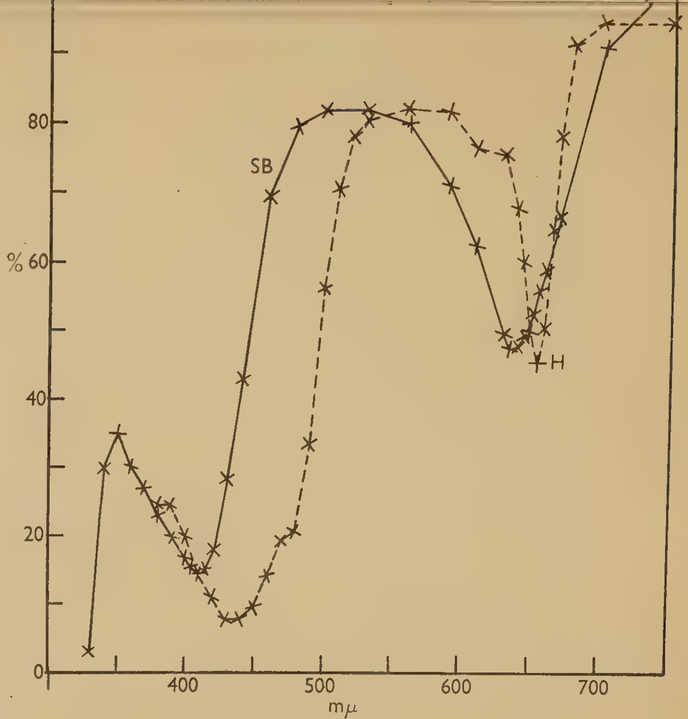


Fig. 3. Percentage transmissions from: SB, an S/20 dilution of the chlorophyll; H, an 80 % acetone extract of a growth of *Hemiselmis rufescens* Parke, developed from water of station E 1, 50 m., 7 January 1952 with a very little *Nitzschia closterium*.

Fig. 4 is made up of plankton extracts, minima 655 and 435 mμ for surface, and 650 mμ only for the very weak 50 m. sample. To avoid turbidity from fine clay and to get a crystal clear solution from the extract it is best to allow the solvent to rise up over the collodion, which rests on the side of a small beaker. After extraction by gentle washing the solvent is drained back into a tube and stoppered. It becomes perfectly clear after a few hours in the dark. Such extracts are the basis of the results shown in Figs. 6 and 7. Fig. 5 illustrates the stability of these solutions if kept in total darkness; after more than 3 years' storage the red absorption band is in each clearly a maximum at 650 mμ, and the violet band at 410 mμ for April and June. The July sample stood a long time before filtering and the extract is obviously of a yellowish tint, which masks this band. Only after a tedious purification is it possible to base quantitative results upon absorption in this region.

The Seasonal Variations in Chlorophyll and their Conversion into Phytoplankton Quantities

Extracts made as explained were carried out for a year, and the results are shown in chlorophyll concentrations per cubic metre in Fig. 6, surface and 50 m., and at intermediate depths when a thermocline existed. These desirable samples were unfortunately not taken in the isothermal March water. The sea temperatures are shown in Fig. 6, for the surface above, and for the bottom just below, the top line of the frame. The thermoclines were found as follows: September, 35 m., 14·0° C.; April, 10 m., 9·7° C.; May, 10 m., 11·7° C.; June, 15 m., 14·0° C.; July, 20 m., 15·8° C.; Aug., 20 m., 15·6° C.

The chlorophyll values given for the water column are based on the mean values for surface and 50 m. samples, and on the assumption that the water was uniform from 50 to 70 m. The latter is taken as the bottom at station E 1, but is actually a depth safe for the water-bottle, with bottom 72–74 m. according to tide and exact position. The chlorophyll present at intermediate depths was read off from Fig. 7, the 50–70 m. amounts being calculated as before. The broken line in Fig. 6 shows the amount of chlorophyll in the column per square metre of surface. The autumn plankton outburst is in late September in an almost isothermal water column—an indication that the deep water mineral nutrients had been rendered available at the well illuminated depths. The winter minimum appears at the very end of November, possibly because December observations were lacking. There is then a rise, steep after February, to a surface maximum in March and a column maximum in April, followed by a tumble down, so that June is the minimum for the year. The observations cease with a rise towards the autumn high. Thus the September and March columns have over 1·0 g./m.^{2} chlorophyll, the April maximum and the June minimum being 1·32 and 0·15 g./m.^{2}.

One may attempt to convert these chlorophyll quantities into weights of

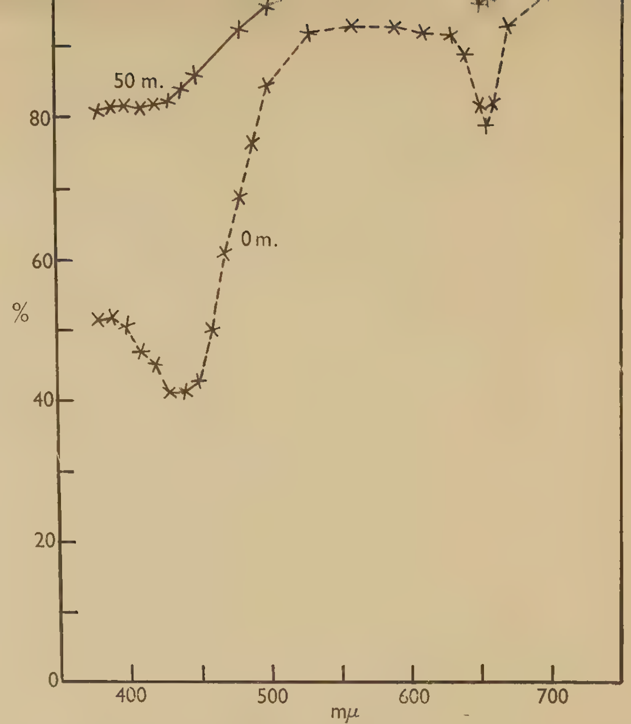


Fig. 4. Percentage transmissions given by extracts from plankton, collodion filtered from 0 and 50 m. 2 l. samples of water from station E1, 11 March 1952.

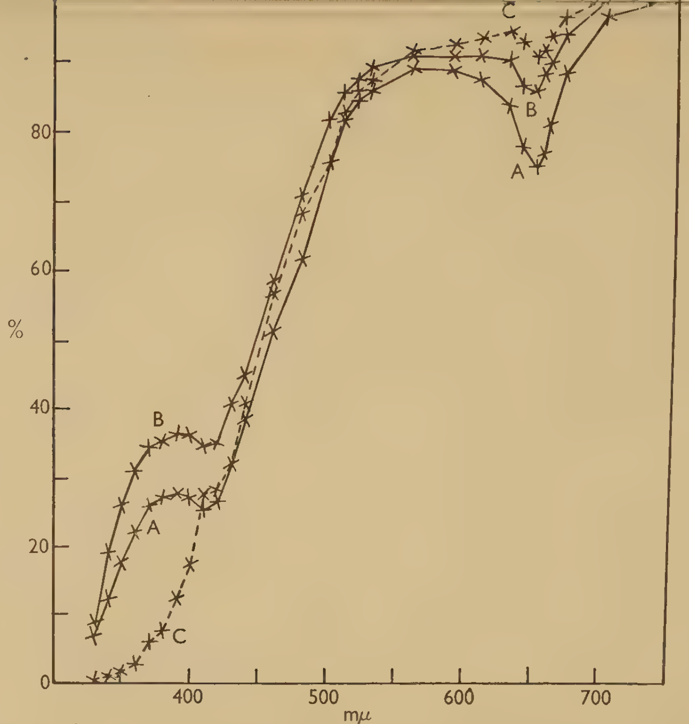


Fig. 5. Percentage transmissions given by extracts qualitatively similar to those of Fig. 4, but from about 20 l. samples through paper using larger volumes of aqueous acetone. Those from station E1, surface, were for April (A), June (B) and July (C), 1949. There was delay in filtering and samples were stored in the dark for over 3 years.

phytoplankton as follows. Riley (1941 *a*) found 2·91 % of chlorophyll in the dry organic matter of his plankton. This is close to Pace's (1941) 2·32 % for the sum of chlorophyll *a* and *b* in *Nitzschia closterium*, in which a lower value is to be expected as the analysis refers to a silica-bearing organism only. Strain (1951), however, quotes unpublished results by Spoehr and Milner showing that *Chlorella* grown in intense light contained chlorophyll equivalent to only 0·03 % of the dry weight of the cells; but in light of low intensity, the same organism produced chlorophyll equivalent to 6 % of the dry weight. Under normal conditions of growth one is probably fairly near the truth in taking Riley's value, based on analysis of plankton. Furthermore, Rohde, when discussing the reliability of extinction coefficient measurements in the quantitative study of an algal culture, concludes with 'if possible, therefore,

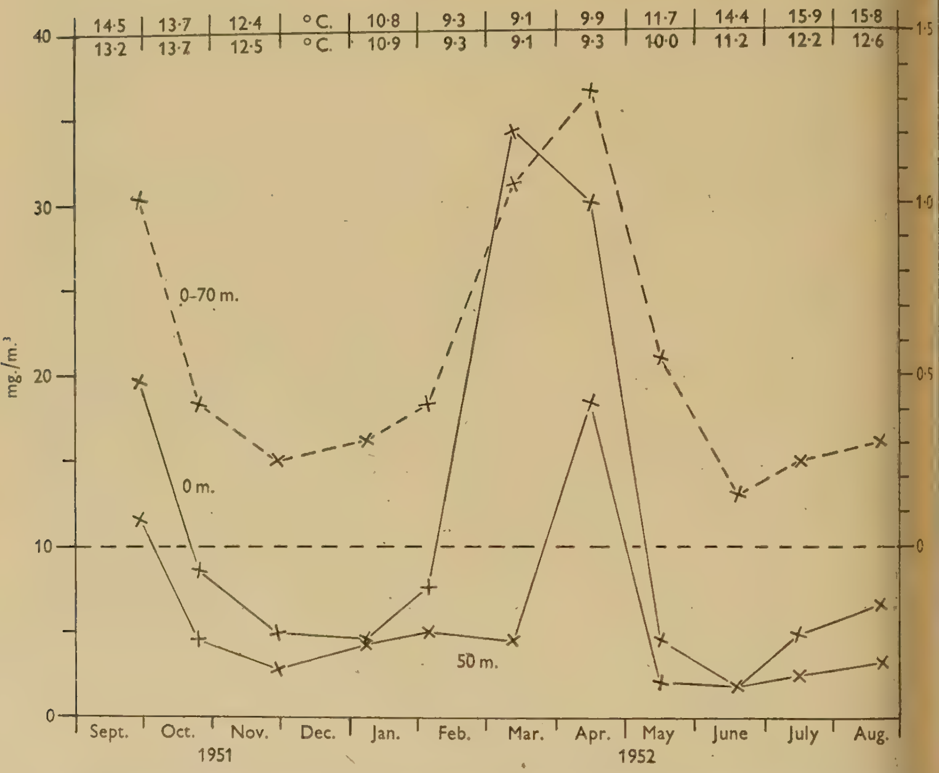
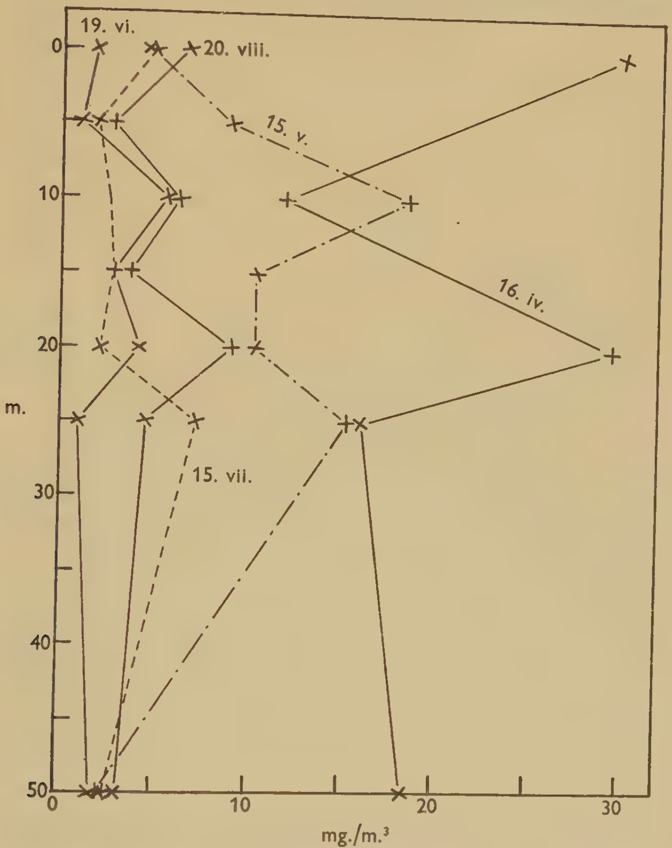


Fig. 6. Monthly variation in chlorophyll values. The left-hand ordinates (for continuous line graphs) denote chlorophyll in mg. obtained from the phytoplankton of 1 m.³ of sea water at station E1 (50° 02′ N., 4° 22′ W.), surface and 50 m. The abscissae show dates of sampling from September 1951 to August 1952 inclusive. The right-hand ordinates (for broken line graph and base) indicate the chlorophyll in the water column, 0–70 m., per square metre, as obtained from data represented in this figure and Fig. 7.

should carry out counts of the cell or colony concentration and determina- of the chlorophyll content in parallel with the photometric determina-’. Taking 2·91% of chlorophyll gives a factor 34·5 by which the weight ry matter may be found from the pigment. To convert to wet weight the weight is assumed to be 20% (Atkins, 1923), as was done in the calculation ne phytoplankton crop from phosphate consumption. When one adds



g. 7. Variation in chlorophyll values (as mg./m.³) with depth of sampling (metres) on five dates in 1952.

her the wet weight of phytoplankton as found for the 11 months, and for nissing December observation takes the mean of November and January, arrives at 1·09 g. as the content in the column. This may be compared the value found from phosphate in 1923, 1·4 g.; and in the less rich water ved more recently Atkins has found for 1948 a consumption, indicating duction, of 84% of the 1923 value, namely 1·17. The actual quantity of phate available in 1948 was only 75% of the 1923 value. Were the utilization less efficient than in 1948 and proportional to the amount available in winter (for which see the discussion in the accompanying paper on phosphate), the sum of the phytoplankton produced as a minimum value would be 1·05 g. This is close enough to the yearly total of the monthly standing crop to suggest that this must be eaten almost as fast as it is produced.

The surface chlorophyll per cubic metre is on the whole greater than that recorded by Atkins & Parke (1951), but the present method appears to be an improvement on that available earlier and the samples were more expeditiously handled. Our values up to 34·2 for March and 30·0 for April 1952 are quite comparable with those of Riley's surface values for Long Island Sound (1941*a*)—17·4 mg./m.^{3} up to 62·0 as maximum—and Georges Bank (1941*b*)—5·6 mean up to a 27·2 mg./m.^{3} maximum.

EXAMINATION OF THE COLLODION 4 CM FILTER DISKS

The filter disks gave a most surprising variety in their intensity. The colour was usually greyish, with black specks and a few fibres visible with a hand-lens, which also showed copepods and medusae. The volume filtered being so small, 1 or 2 l., it is not considered that the numbers observed can give an accurate idea of their depth distribution, but in June one medusa per litre was found at 15 m. and five at 20 m.—in July one only at 15 m. No copepods were found above 10 m. In June, July and August at 10 m. we got 1, 0, 1; at 15 m., 7, 0·5, 2; at 20 m., 6, 4·5, 5; at 25 m., 8, 3·5, 0·5; and at 50 m., 5, 3, 0·5. The monthly totals were 27, 11·5 and 9. Such a distribution can have nothing to do with the thermocline or temperature gradient, and is probably influenced by light. The transparency of the water, indicated roughly by the depth of visibility of the Secchi disk, was for these months respectively 23, 18 and 11 m. The remarkable clearness of the water in June is perhaps related to the collection of the mud-coloured suspended matter in the surface sample, the deeper samples showing very little, even allowing for the fact that save for the surface and the 50 m. (2 l.) they were 1 l. each (Fig. 8). Fig. 8 also shows the series of 2 l. samples of 15 July. In these the surface and 50 m. are rather similar, and 25 m. shows up as darkest in the photograph. Visually it was not so but had a yellowish tint, as had also 50 m. to a lesser extent.

THE BOTANICAL COMPOSITION OF THE PHYTOPLANKTON AT STATION E1

This station has been visited for many years and records of its phytoplankton have been published in the *Bulletin Planktonique* up to 1912 and summarized later by Ostenfeld (1931) but the following observations may be of interest. E1 is 10 miles S.W. of the Eddystone and the list published by Harvey,

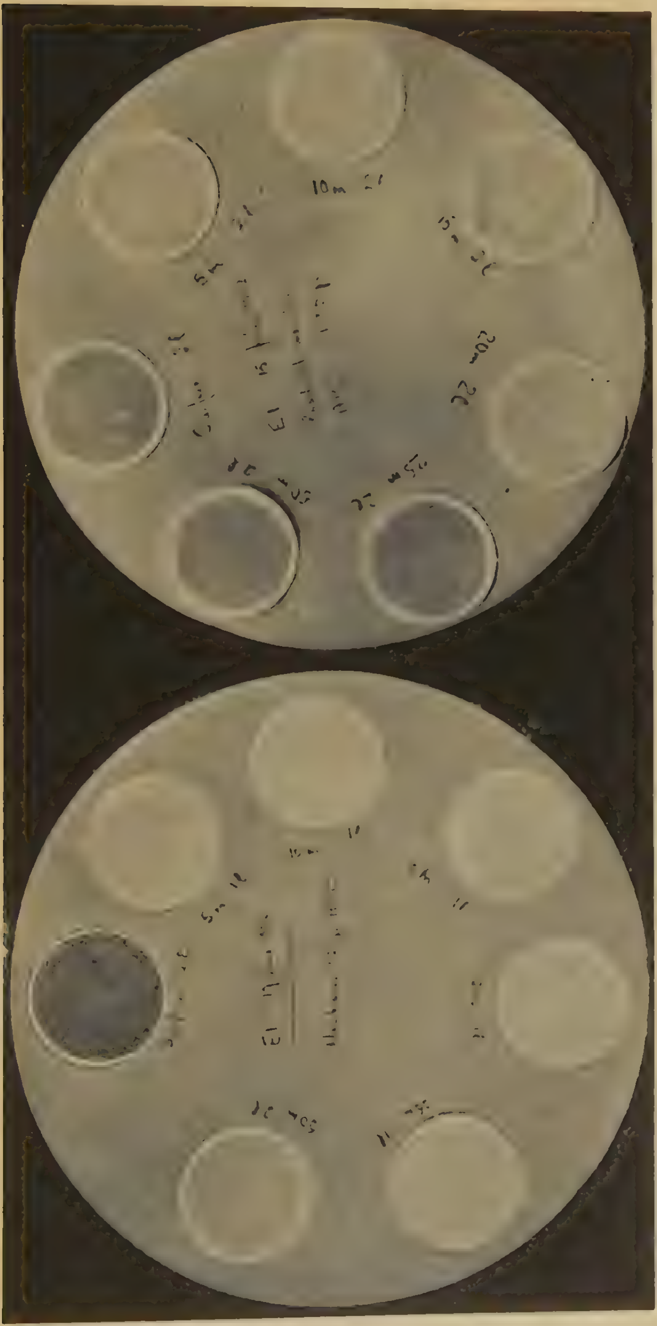

10m
20m
25m
30m
5m

Fig. 8. View of the collodion filter disks with collected matter. Series for 19 June (left) and 15 July 1952 (right).

Cooper, Lebour & Russell (1936) concerns station L4, depth 50 m. and 14 miles nearer shore, about 5½ miles from Plymouth breakwater. We had no plankton hauls and no time to count the algae in the filter disks, but a qualitative examination was made as follows. Samples of the water, 100 or 250 ml., were placed in conical flasks enriched with Miquel's solution and exposed in a south window in winter or a north window in late spring and summer. Some, but probably not all, of the phytoplankton multiplied rapidly, and periodical examinations were made. The plus signs in Tables I and II are an attempt to indicate the relative amounts found in the earlier stages of the cultures; +, present; + +, more frequent; + + +, plentiful; + + + +, very plentiful. Though sampling was begun in September it was only, unfortunately, on 8 February 1952 that the enriched samples were exposed in the window; before that the Winchester bottles had diffuse light in a south room. The earlier dates are probably too sparsely represented. No attempt has been made as yet to represent the depth distribution.

Table I shows the Bacillariophyceae found, twenty species, of which *Nitzschia closterium*, *Melosira Borreri* and *Navicula* sp. were the most regular in occurrence. As compared with the L4 list of 1935 it is remarkable that *Nitzschia closterium* then appeared in negligible amount in March, April and September. *N. delicatissima* and *N. seriata* appear on both lists. *Paralia sulcata*, common at L4, was only found once at E1. *Guinardia flaccida*, which occurred regularly at L4, was absent from E1, as were also over sixteen other species. Found at E1, but not mentioned for L4, were *Fragilaria* sp., *Grammatophora marina*, *Melosira* sp., *Rhizosolenia alata* f. *genuina* and *R. hebetata* f. *semispina*.

Table II lists the Chlorophyceae, Chrysophyceae, Cryptophyceae, Dinophyceae and Miscellaneous found as for Table I. None of the Chlorophyceae are recorded in the L4 list, yet in our cultures they were the striking feature in most of the April—August samples, save the 0 m. samples, the 5 m. for April and the 50 m. sample for July, which were brown with diatoms; also, the 20 m. July sample had a very minute bacterium (with one flagellum visible) which produced a pink colour, modified to buff by a Cryptomonad. This organism became conspicuous in the 10 m. sample later. In several of the series 20 m. was noticeably behind the others in development.

Of the Chrysophyceae, *Phaeocystis* was recorded from L4 and has often been found at E1, but it was absent from our samples. Two others, however, were present at E1 though not at L4: *Coccolithophora* sp., very plentiful in July and August samples, and a member of the Chrysomonadaceae.

The Cryptophyceae afforded two records, in July a species of the Cryptomonadaceae and most remarkably from 50 m., of 7 January, we obtained an almost pure growth of *Hemiselmis rufescens* Parke, which was identified by its appearance and the scarlet pigment insoluble in 80% acetone which was left on the collodion. Its chlorophyll with possibly other pigments gave the curve

TABLE I. ALGAE IDENTIFIED IN CHEMICALLY ENRICHED SAMPLES.

BACILLARIOPHYCEAE:	27. ix. 51	24. x. 51	7. i. 52	5. ii. 52	11. iii. 52	16. iv. 52	15. v. 52	19. vi. 52	15. vii. 52	20. viii. 52
Asterionella japonica Cleve & Möller*	.	.	.	.	++	.	++	.	.	++
Cerataulina Bergonii H. Pérag.†	.	.	.	.	.	.	.	.	.	+
Chaetoceros sp.	.	.	++	.	.	.	.	.	+++++	.
Coscinodiscus sp.	+	.	.	.	++	.	.	.	.	.
Fragilaria sp.	.	.	.	.	.	++	+	.	+	.
F. oceanica Cleve	.	.	.	.	.	.	.	.	.	++
Grammatophora marina Kütz.	.	.	.	.	.	.	.	.	.	+
Leptocylindricus danicus Cleve	.	.	.	.	.	.	.	.	+++	++
Melosira borreri Greville	++	++	.	.	++	++++	+++	++	.	+
Navicula sp.	.	.	.	++	+++	++++	++++	++++	+++	++
Nitzschia closterium Ehr.	+++	+++	++++	+++	+++	++++	+	+++	++++	+++
N. delicatissima Cleve*	.	.	.	.	.	.	.	.	.	++++
N. seriata Cleve†	.	.	.	.	.	.	.	.	.	++
Paralia sulcata Ehr.‡	.	.	.	++	.	.	.	.	.	.
Rhizosolenia alata Brightw. f. *genuina* Gran.‡	.	.	.	.	.	.	.	.	+	+++
R. f. *indica* Péragallo	.	.	.	.	.	.	.	.	.	+
R. hebetata (Bail.) f. *semispina* Hensen‡	.	.	.	.	.	.	.	.	.	++
Skeletonema costatum Greville‡	.	.	.	.	++	.	+	.	++	+++
Thalassiosira gravida Cleve	.	.	++	.	+++	++	.	.	.	.
Thalassiothrix (*Thalassionema*) *Nitzschiodes* Grun.‡	.	.	.	.	.	.	.	.	.	+

* For southern part of North Sea or Flemish area.
† Indicates species similarly listed for eastern part.
‡ Indicates species listed by Ostenfeld (193.) as common in western part of English Channel.

September, October, January and February samples enriched and exposed in window, 8 February 1952; the same holds for Table II.

Table II. Algae identified in chemically enriched samples.

	27. ix. 51	7. i. 52	5. ii. 52	11. iii. 52	16. iv. 52	15. v. 52	19. vi. 52	15. vii. 52	20. viii. 52
CHLOROPHYCEAE:									
Chlamydomonas sp.	.	.	.	++	.	.	.	.	.
Chorella sp.	.	.	.	++++	++++	++++	+++	++	+
Endoderma sp.	.	.	.	+++	++++	+	+++	.	.
Halosphaera viridis Schmitz	+	.	.	.	.	.	.	.	.
Platymonas tetrahele West.	.	.	.	.	.	.	.	++	.
Pyramimonas sp. Schmarda	.	.	.	+++	.	++	.	.	.
Stichococcus bacillaris Naeg.	.	.	.	+++	++++	+++	+++	++	.
Ulothrix subflaccida Wille	++	.	+	.	.	.	.	.	.
CHRYSOPHYCEAE:									
Chrysomonadaceae sp.	.	.	.	.	.	.	.	++	.
Coccolithophora sp.	.	.	.	.	.	.	.	++++	++++
CRYPTOPHYCEAE:									
Cryptomonadaceae sp.	.	.	.	.	.	.	.	++	.
Hemiselmis rufescens Parke	.	++++	.	.	.	.	.	.	.
DINOPHYCEAE:									
Prorocentrum micans Ehr.	.	.	.	.	.	++++	.	.	.
MISCELLANEOUS:									
Flagellates, colourless	.	.	.	.	.	++	.	++++	++++
Amoebae	.	.	.	.	.	.	+++	.	++
Ciliates, colourless	.	.	.	.	.	++	++++	++	++
Bacterium, pink	.	.	.	.	.	.	.	++++	.

n in Fig. 3. Of the seven Dinophyceae in the L4 list only *Prorocentrum* *s* was observed in the E1 cultures.

desire to express our indebtedness to Miss D. Ballantine, Dr M. V. ur, Dr T. J. Hart and Dr M. Parke for much help in the identifications, our thanks to Mr F. A. J. Armstrong for filtering the samples and for erature observations and to Mr A. E. Stoate and Mr A. Mattacola for the graphs. Finally, for these and many other sea-water samples and Secchi observations we have pleasure in thanking Lieut.-Comdr. C. A. Hoodless, C., R.N.R., and the crew of the R.V. *Sabella*.

SUMMARY

rophyll from the phytoplankton at station E1 was examined from ember 1951 till August 1952, from 0 to 50 m. The minimum was in June, g./m.^{3}, and the maximum 34·2 in March, both surface samples. The num 50 m. sample contained 18·4 mg./m.^{3} in April. The winter minima 4·6 and 4·3, surface and bottom, in November. Converted to water nn (70 m.) values, over 1·0 g./m.^{2} is obtained for the wet weight of plankton for September and March, the April maximum and the June num being 1·32 and 0·15 g./m.^{2}. A comparison with the production ned from phosphate analyses suggests that the phytoplankton crop each h is rapidly devoured.

act proportionality was found between concentration and absorption in rophyll band up to 4·0 mg./l. and a moderate error up to 8·0 mg./l. The ral absorption curves have been given for pure cultures of phytoplankton or cells filtered out of sea water. The chlorophyll in such extracts, made 80 % acetone, is stable when kept in total darkness.

e collodion disks containing suspended clay and the algal cells may show prising variation in their colour intensity, from dark grey to a very faint even though obtained from sea water about 20 miles from land and over in depth. The surface may be far darker than 5, 10 or 15 m. samples.

e botanical composition of the water was studied by allowing the algae ltiply in diffuse light after enriching the water chemically. Eight species lorophyceae, and one species each of the Chrysophyceae and Dino-ceae were recorded. Of the Cryptophyceae one winter sample from 50 m. a nearly pure growth of *Hemiselmis rufescens* Parke. The diatoms *ira borreri*, *Nitzschia closterium* and *Navicula* sp. occurred commonly.

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THE PRESERVATION OF FISHING NETS, TRAWL TWINES AND FIBRE ROPES FOR USE IN SEA WATER

By W. R. G. Atkins, F.R.I.C., F.R.S. and F. J. Warren
The Plymouth Laboratory

paper is a continuation of our publication, in 1941, of exposure tests upted by the war. As no sea-water basin was available till recently the was delayed, and in the post-war confusion several mixtures intended ial unfortunately got mislaid. During the war, however, one of us d water-absorption tests which have proved interesting and appear to re useful for work in the sea than the British Standards Institute tests, extend only to 6 hr. immersion; this is far too short a period for some ses.

ABLE I. WATER ABSORPTION TESTS ON SISAL ROPE IN SEA WATER

The numbers represent percentage absorbed.

ebruary 1943	Immersion period (hr.)					Diameter (cm.)	
	0·5	5	24	48	96	Initial	Final
ntreated	53	53	54	58	59	8·2	8·6
nulsion treated	.	28	55	52	.	9·4	10·2
aterproofed	.	12	26	38*	.	8·7	9·8
Above repeated after 22 days air-drying, till constant							
ntreated	.	.	.	38	64	8·6	8·8
nulsion treated	.	.	.	48	54	9·8	9·9
aterproofed	.	.	.	39	42	10·2	10·2

Percentages here are calculated on the dry weight after immersion.
* 72 hr. All 6 ft. with ends bound with hemp string.

WATER ABSORPTION TESTS

I shows the absorption of water by sisal rope—the only fibre then ble—untreated and after commercial treatments with emulsion and roofing. The emulsion treated was indistinguishable from the un- d after 1 day, whereas the waterproofing still showed a lesser uptake days. But with a second immersion the differences varied irregularly. le II records the percentage uptake of Cuprinol and of tar by manila, nd coir ropes 3 in. in circumference, also the uptake with commercially roofed manila and sisal. Dry weights of preservative are also shown and ight of preservative remaining after drying and slow draining, calculated ercentage of the wet weight after treatment. There is little difference between the final water uptake of manila and sisal, but the 'waterproofed' ropes ultimately absorb more water than the untreated, and the same holds for preservatives. Coir takes up more than twice as much water as the other two. But the treatment of manila, waterproofed manila and sisal with the preservatives fails to make the ropes heavy in water, for the water absorption is reduced by approximately the weight of the preservative taken up. There

TABLE II

Sample no.	Treatment, May 1950	Preservative wet wt. (%)	Preservative dry wt. (%)	Preservative residue (%)	Sea water absorbed (%)		Water-preservat % on untreat
					1 day	15 days	
1	Manila 3 in. dry	.	.	.	.	.	.
2	Do. untreated	.	.	.	35	45	45
3	Do. Cuprinol	11	3	28	31	41	44
4	Do. tar	21	17	84	14	29	46
5	Manila 3 in. waterproofed, dry	.	.	.	.	.	.
6	Do. untreated	.	.	.	26	48	48
7	Do. Cuprinol	13	6	45	28	44	50
8	Do. tar	21	18	84	16	34	51
9	Sisal 3 in. dry	.	.	.	.	.	.
10	Do. untreated	.	.	.	37	44	44
11	Do. Cuprinol	16	7	42	34	40	46
12	Do. tar	26	23	87	15	26	49
13	Sisal 3 in. waterproofed, dry	.	.	.	.	.	.
14	Do. untreated	.	.	.	47	55	55
15	Do. Cuprinol	22	11	50	43	52	63
16	Do. tar	38	33	87	17	31	64
17	Coir 3 in. dry	.	.	.	.	.	.
18	Do. untreated	.	.	.	88	117	117
19	Do. Cuprinol	49	31	63	77	89	120
20	Do. tar	79	72	90	49	75	147

is some increase with waterproofed sisal when tarred. In the foregoing the ends of all ropes were whipped as usual with hemp twine, but were not sealed, as this is not customary in fishing vessels. Samples shown as 'untreated' means no further treatment after receipt from the factory. The tar treatment was cold immersion for 48 hr. (31 May 1950) by courtesy of the Plymouth branch of the Gourock Ropework Co. The netting was allowed to drain for 1 day. Cuprinol samples were immersed for 18 hr. (30 May 1950) and weighed wet after 4 hr. draining; they were then air dried till constant in weight after 47 days, and placed in sea water 18 July 1950, till saturated.

Table III shows the results of tap water absorption tests on the untreated dry controls of Table II. Duplicate tests were made in April 1952, the ends being also sealed, with Berry's bitumastic rubber compound. The B.S.I. test (1946, no. 908) specifies for waterproofing 1 hr. immersion, wiping dry and immersion for 5 hr. more. Though a 6 hr. test shows benefit from waterproofing and a decreasing effect is shown for 1, 2 and perhaps 3 days, with r immersion no difference is apparent. With so much water and pre- tive held in the interstices it is obvious that exact percentage duplications ot to be expected, but the absorptions of the untreated fibres agree nably well in the various tables.

TABLE III. WATER ABSORPTION TESTS ON ROPES

.S.I. 1946 No. 908 specification but continued longer. April, 1952. The numbers represent percentage absorbed.)

mple o.	Sample	Immersion period (hr.)						
		1	6	24	48	72	120	144
1	Manila 3 in.	28	35	41	45	49	51	51
5	Do. waterproofed	8	18	29	38	43	49	49
9	Sisal 3 in.	39	40	44	45	48	49	48
3	Do. waterproofed	12	19	29	40	47	53	52
7	Coir	90	86	108	110	120	126	120

DURABILITY TESTS

ifficult to devise an exposure test which shall be of real value as indica- f usefulness on nets, trawl twines and ropes. One can only arrange the ods in order under the conditions of test selected or imposed by nature.

on ropes in Plymouth Sound, on the old Hoe pier, now destroyed, were ble, as the polluted water was a good sample of harbour conditions and ion in fishing gear. The water in the basin at Pier Cellars is far purer. nes, however, fouling occurs from drifting seaweed. Though samples t there be stolen, they can be washed away in heavy weather. The tests to become a measure of the solubility of the preservative, as against for the untreated specimen far longer than under harbour conditions.

the present series the best of the earlier treatments, see Atkins & Purser), copper naphthenate (Cuprinol), and the quite good treatment—save pes—recommended by Dr Olie were tested against a few others. Olie's d (see Atkins, 1936) is cheap and easily applied and many millions of bags were thus preserved during the war.

e specimens were suspended in the basin so as to be out of the water for . every tide. In spite of what seemed secure fixing most of one set of twines and one entire set of ropes were washed away.

e specimens were inspected once a fortnight or once a month and were by hand.

cotton netting aluminium stearate in petrol did surprisingly well licate tests, and ranked with the copper soaps, the best of which lasted ths, but Olie's treatment was so near as to have advantages on general ds. On the trawl twines the British Columbia grade of Cuprinol was the best, and lasted 16 months. Among the ropes the four Cuprinol samples, including two diluted with Coalite oil, low-temperature distillation, an American ammoniacal copper and novenate copper in petrol are still under test after 16 months.

TABLE IV. DURABILITY OF SAMPLES IMMERSED IN SEA-WATER BASIN, NEAR CAWSAND, PLYMOUTH SOUND

Sample no.		Cotton netting (%)	Trawl twines (%)	Sisal rope (%)
1	Untreated: kept in dark in laboratory	•	•	•
2	immersed	100	100*	100
3	Cuprinol: standard grade	167	121	++
4	British Columbia grade	162	183	++
5	Copper N.H. 8	128	138	++
6	Aluminium stearate: 1 lb. to 1 gal. benzole	134	100*	100
7	1 lb. to 1 gal. Coalite oil	128	106*	123
8	1 lb. to 1 gal. petrol	162	100*	100
9	Novenate copper: 1 lb. to 1 gal. benzole	162	121	129
10	1 lb. to 1 gal. Coalite oil	145	106	127
11	1 lb. to 1 gal. petrol	150	106	++
12	Cuprinol: with equal volume Coalite oil	133	121	++
13	1 vol. with 3 vol. Coalite oil	150	106	++
14	Olie's treatment	150	106	123
	Life of untreated in days	164	265	382

Samples immersed in Pier Cellars tidal basin 21 February 1951.

Cotton 14 S/6-ply, white, 2½ in. mesh. Mean of duplicate sets.

Trawl twine, manila, 3-ply, 150 yd/lb. Duplicate set washed away 9 January 1952; only specimens showing result of duplicate tests have * mark. Sisal rope was $\frac{7}{8}$ in. six thread.

One set of ropes washed away, 26 February 1952, before any failed; sound after 493 days marked ++.

We are indebted to the courtesy of the Gourock Ropework Co. and Messrs Hawkins and Tipson for ropes; to Messrs Cuprinol, the Coalite Low Temperature Carbonization Co., Messrs Boake Roberts and the Nuodex Products Co., New Jersey, U.S.A., for preservatives; also to Mr A. E. Stoate for much help throughout the work.

SUMMARY

A trade waterproofing of sisal rope had still some effect after 3 days in sea water, the untreated being four-fifths saturated in 1 hr.

Treating manila, sisal and coir rope with Cuprinol or tar increases the wet weight at the most only slightly as less water is taken up than without preservative.

Water equilibrium is not attained till long after the B.S.I. specification test period for waterproofing sisal rope, but some effect of waterproofing may be distinguished up to perhaps 3 days.

On cotton netting copper naphthenate preservatives were most durable in a clean sea-water basin, but Dr Olie's method with cutch and ammoniacal copper sulphate ran them close and is cheaper. The best lasted 9 months as against untreated 5½ months.

trawl twines the British Columbia grade of Cuprinol, a proprietary ure containing copper naphthenate, was the best of those tried, and the es lasted 16 months as against almost 9 for untreated manila.

thin sisal rope six copper preparations are still under test.

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OBSERVATIONS ON *NUCULA TURGIDA* MARSHALL AND *N. MOOREI* WINCKWORTH

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Station, Millport

(Plate I and Text-figs. 1–8)

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INTRODUCTION

1950 a study of the specific differences and growth rates of the British ecies of *Nucula* (Mollusca, Protobranchiata) was commenced. Winckworth 932) lists five species which, with the exception of *N. turgida* and *N. moorei,* n be readily distinguished from each other. The present observations on er 2500 specimens of *N. turgida* and *N. moorei* show that the diagnostic aracters differentiating the two species are not valid, and as no new differces have been found it is proposed that they should be recombined as one ecies, *Nucula turgida* Marshall 1875, this being the first available name.

Until 1930, *N. turgida* and *N. moorei* were considered to be one species der the name *N. nitida* Sowerby. Then Winckworth (1930) pointed out that e name *N. nitida* was preoccupied and proposed *N. nitidosa* instead. Two pers by Leckenby & Marshall (1875) and Marshall (1893) had been overoked in which a dark unrayed form of *N. nitida* was described as variety *rgida* and a form having coloured rays as variety *radiata*.[1] Jeffreys (1879) scribed another variety under the name *ventrosa* from the Mediterranean *orcupine* Expedition, 1870); this was considered by Marshall (1893), hower, to be his variety *turgida*. After examination of Marshall's specimens om the Dogger Bank and from the work of Moore (1931 *a*, *b*) on the faeces the rayed and unrayed forms, Winckworth (1931) considered the two varieties (*turgida* and *radiata*) distinct and renamed them *N. turgida* Marshall, and *N. moorei* Winckworth. The two species were diagnosed as follows:

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[1] Not to be confused with *N. radiata* Hanley which is now known as *N. hanleyi* Winck-
rth.

Nucula turgida

Shell glossy, with olive-green periostracum, somewhat inflated, anterior margin distinctly prominent, posterior margin evenly rounded; growth lines form distinct wrinkles on the anterior third and the extreme posterior. Faecal pellets subcircular in section, with seven grooves.

Nucula moorei.

Shell usually more glossy, with a pale green periostracum, streaked with coloured rays (usually obvious but exceptionally they may be only just visible), anterior margin almost straight, posterior margin arched; growth lines much finer and wrinkles slight. Faecal pellets quadrate oval in section, with five grooves.

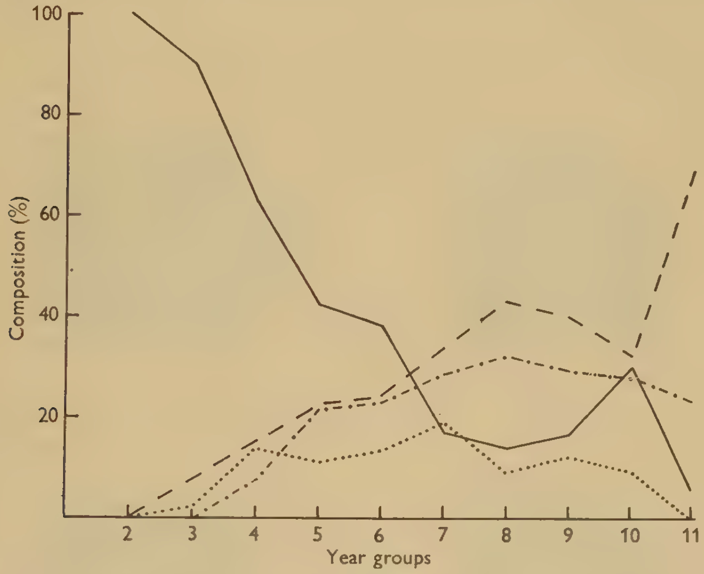
N. nitida is a widely distributed species ranging from Sweden to the Mediterranean. Forbes & Hanley (1853) and Jeffreys (1865) state that it can be taken in sand and sandy silt at low spring tides and at depths ranging from the latter mark to 86 fathoms (157 m.) Both radiate (*N. moorei*) and non-radiate (*N. turgida*) forms are present together throughout the Clyde Sea Area, but there is no record of them being taken at low-water mark. In a survey of the sublittoral fauna in 1949 and 1950 they were taken from sandy silt at 10 m. to fine mud at 180 m. The densest populations occurred in muddy silt (80–90 m. depth) in company with *Corbula gibba*, *Abra alba*, *Cuspidaria cuspidata* and *Nucula sulcata.* The material for the present investigation was obtained from the fauna survey and additional large samples from the Cumbrae Deep off the east coast of Bute and from the Minnard Narrows, Loch Fyne.

SHELL MARKINGS

The colour of the shell has been variously described as pale green, olive-green and yellow. The ground colour of the periostracum of specimens from the Clyde Sea Area is yellow, on which purple-grey markings may be superimposed. These markings can completely mask the yellow ground giving the non-radiate dark form which Marshall called the variety *turgida*. The purple-grey colour is not always laid down continuously and banded forms are found with the bands running parallel to the lines of growth. It can also take the form of radiations at right angles to the lines of growth. These latter forms vary from those that are heavily radiate to those in which the radiations are hardly visible (Plate I, figs. 1, 2). These markings, also, may not be laid down continuously, being often limited to bands parallel to the lines of growth.

Examination of the specimens obtained showed that it was possible to group them into (1) those with markings, and (2) those without markings. Group (1) was then further divided into (*a*) those in which radiate markings could be seen with the naked eye, and (*b*) those in which the markings were

10t obviously radiate. On microscopic examination group (*b*) was again livided into (i) those that were probably radiate, and (ii) those which were orobably non-radiate (Pl. I, fig. 2). The group that was probably non-radiate ncluded the dark non-radiate form and the banded non-radiate form. In a arge sample of over 1400 specimens dredged from the Cumbrae Deep 35·3 % were found to be non-radiate, 12·4 % probably non-radiate, 29·5 %



ext-fig. 1. Percentage composition of the year groups. —, non-radiate; ···, probably non-radiate; —·—, radiate; — —, probably radiate.

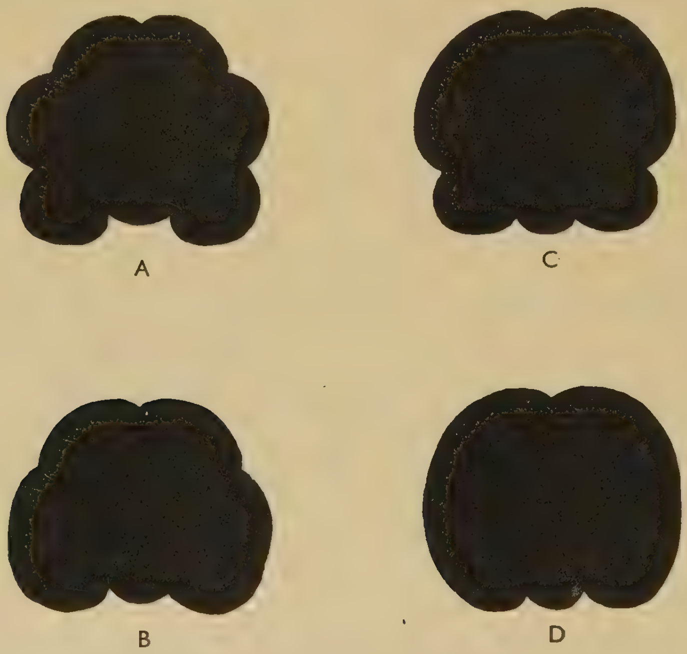
robably radiate and 22·8 % radiate. It was found possible to make a graded eries ranging from those which showed complete purple-grey coloration to 1ose without markings.

This sample shows that the purple-grey markings are not visible until the 1imal is approximately 3 mm. long (in its third year) and that the first clearly ıdiate specimen is 3·6 mm. long. This is shown in Text-fig. 7 in which ıdividual length is plotted against numbers in the four groups. In larger 1ells with purple-grey radiations there is an area in the region of the umbo rresponding to early shell growth that is not marked. Text-fig. 1, which ots the percentage composition for each year group of this population, shows early that the number of shells with markings increases with age.

Examination of the growth lines shows a considerable amount of variation both radiate and non-radiate forms. In both, distinct wrinkles may be rmed on various parts of the shell, but this feature could not be used for issification.

Faeces

Moore (1931 *a*, *b*) points out that the form of the faecal pellets is of value in the specific identification of various molluscan families. He describes their form in the British species of *Nucula* and differentiates the non-radiate form from the radiate form by this means. He states that the former has seven grooves and the latter five grooves (Text-fig. 2, A and B). The faeces of the two forms were re-examined. Freshly caught specimens were placed separately in vessels of clean water and the faecal pellets examined. In addition, transverse sections were cut through the rectal region (Table I; Pl. I, fig. 3).



Text-fig. 2. Faecal pellet types (end view); A, non-radiate form (Moore); B, radiate form (Moore); C, and D, other forms.

It will be seen from Table I that the present observations do not agree with those of Moore. Of fifty-six specimens examined, fifty-one had the non-radiate type of faeces (seven grooves) and only three had the radiate type (five grooves). Only one of these three corresponds to an animal with radiate shell markings and one to a shell that was probably radiate. Pl. I, fig. 3, is a photomicrograph of a transverse section of the rectum of an animal with a radiate type of shell. This shows a faecal pellet with seven ridges which Moore states is found in the non-radiate form. In addition, two other variations in the form of the faecal pellets were noted (Text-fig. 2, C and D).

TABLE I

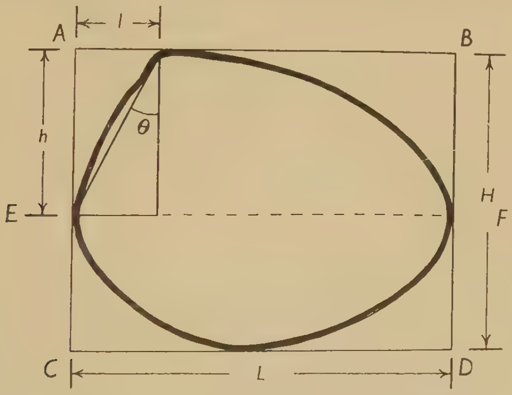
Shell markings	Number examined	Faecal pellets		
		Radiate form	Non-radiate form	Other forms
Radiate	14	1	12	1*
Probably radiate	7	1	6	—
Non-radiate	20	1	18	1†
Probably non-radiate	15	—	15	—
Totals	56	3	51	2

* See Text-fig. 2C. † See Text-fig. 2D.

Although the two forms as described by Moore exist, it is clear from this amination that they cannot be used as a means of differentiating the two ecies.

SHELL MEASUREMENTS

easurements shown in Text-fig. 3 were taken for all British species of *ucula*. The shell was placed on a grid (*ABCD*) divided into squares of side mm. so that a line through the points *E* and *F* was parallel to the lines *AB*



Text-fig. 3. Diagram to show the shell measurements taken. For explanation of the symbols see text.

d *CD*. The measurements were taken with the aid of a travelling microope the magnification being ×8. The measurement *W*, the greatest width the shell (pair of valves), was also taken. The measurements *h* and *l* were ken so that the angle θ could be calculated, as it was found that this angle ffered in the different species of *Nucula*. In addition, neglecting the curvature the shell margins, the values l and $L-l$ and h and $H-h$ define the outline of e shell and therefore are likely to be the best values from which discriminant nctions can be calculated. Comparisons have been made mainly from the easurements of definitely radiate and non-radiate forms, particularly those om the large sample taken from the Cumbrae Deep.

Comparison of the radiate and non-radiate forms in which mean measurements for height (*H*) and width (*W*) were plotted against length (*L*) show that, except for high shell measurements where numbers are low and the variations in shell shape are the greatest, there is no significant difference between the two forms. Further comparisons of the mean shell measurements of each year group indicate even more strongly that there is no difference between the radiate and non-radiate forms even in the older age groups (Table II, Text-figs. 4 and 5). In addition, it is seen that the variations themselves show identical ranges. It would be possible to obtain identical curves even though the angle θ differed in the two forms. From Table II it is seen that the shell angle does not so differ.

TABLE II

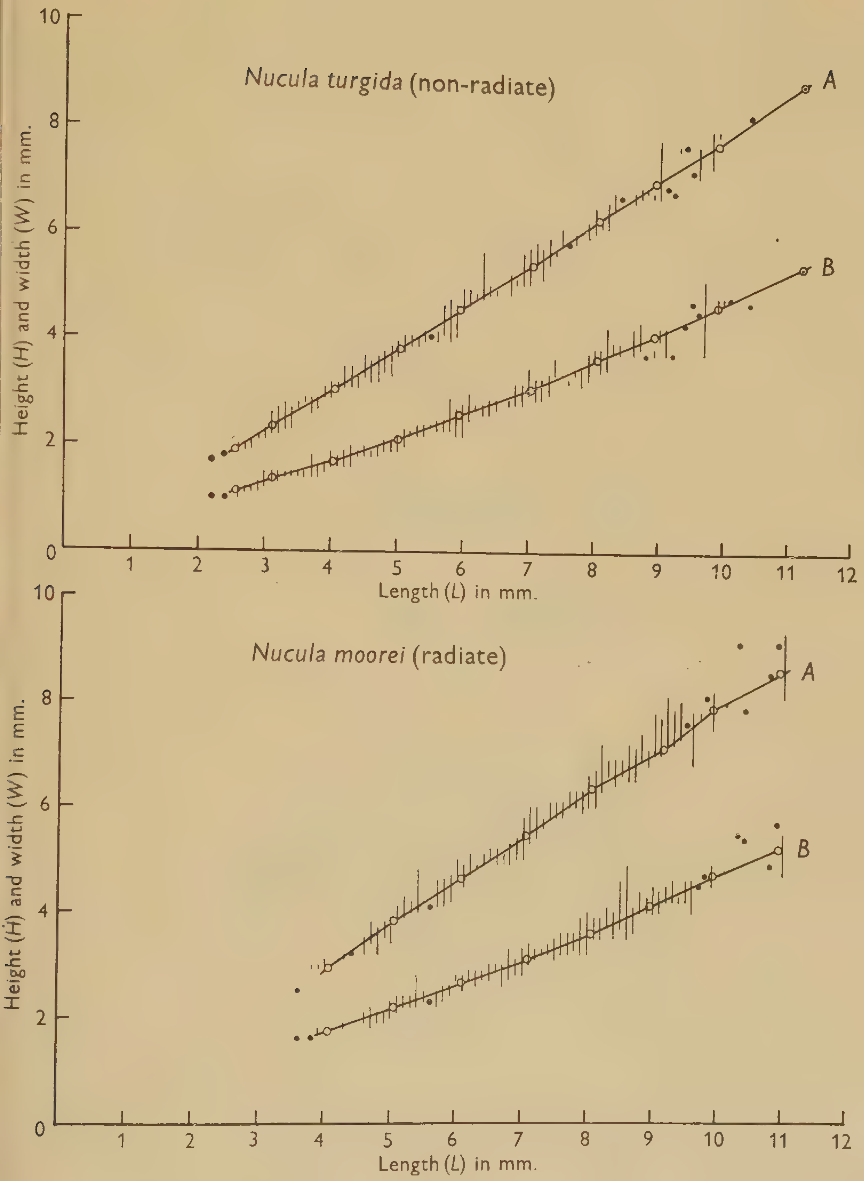
Year group	*L*		*l*		*H*		*h*		*W*		θ		Nos./yr. group	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
1	2·55	—	0·70	—	1·90	—	1·01	—	1·10	—	34° 43′	—	11	—
2	3·11	—	0·83	—	2·33	—	1·21	—	1·33	—	34° 27′	—	81	—
3	4·04	4·05	0·98	0·98	3·05	2·94	1·65	1·69	1·68	1·74	30° 43′	30° 07′	109	13
4	5·02	5·04	1·11	1·09	3·82	3·84	2·06	2·06	2·12	2·17	28° 19′	28° 53′	107	54
5	5·97	6·08	1·32	1·35	4·60	4·63	2·49	2·54	2·58	2·65	28° 04′	28° 01′	99	61
6	7·04	7·05	1·56	1·63	5·46	5·47	3·04	3·04	3·09	3·10	27° 10′	28° 12′	47	78
7	8·05	8·04	1·87	1·83	6·35	6·33	3·61	3·55	3·69	3·63	27° 23′	27° 16′	35	82
8	8·92	8·94	1·99	1·98	7·09	7·12	4·00	4·06	4·14	4·12	26° 27′	26° 00′	23	41
9	9·88	9·90	2·15	2·23	7·81	7·91	4·42	4·63	4·72	4·73	29° 00′	25° 41′	13	12*
10	11·20	10·93	2·30	2·60	9·00	8·60	5·10	4·73	5·50	5·20	24° 17′	28° 48′	1	4*

Comparison of shell measurements (in mm.) of (*A*) non-radiate and (*B*) radiate forms.
* Discrepancies due to the small number of specimens in the year groups.

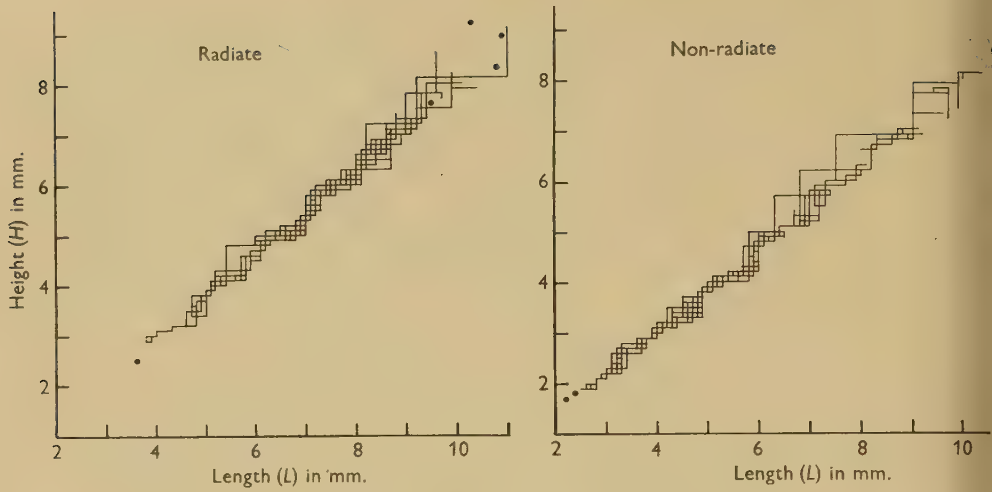
In the list of diagnostic characters given by Winckworth the curvature of the shell margins is said to differ in the radiate and non-radiate forms. No such distinction could be observed although the shell outlines were compared.

As there is no significant difference between the shell measurements and no difference in the shell outline of the two forms, it is not necessary to calculate discriminant functions. Values of t calculated for L, H, W, l and h are 1·59, 0·47, 0·97, 0·87, and 1·34 respectively. All these are considerably lower than the value 2·447 for t (corresponding to a probability $P = 0{\cdot}05$) given in Fisher & Yates' (1948) tables. Therefore the means of the values in the radiate and non-radiate groups do not differ significantly from one another.

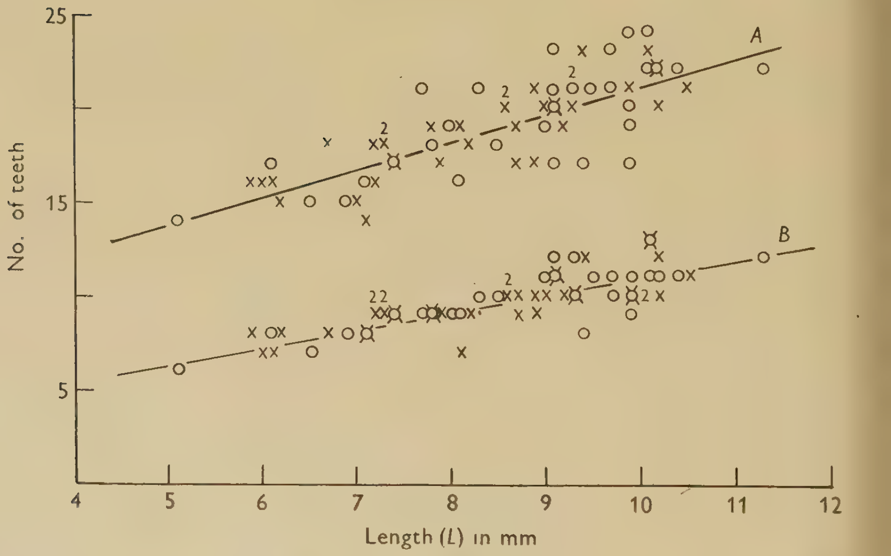
In addition to the measurements listed above the numbers of hinge teeth in the two forms were compared (Text-fig. 6). Although there is a considerable variation in the numbers of teeth in each form for a particular shell length, comparison of the numbers of anterior and posterior teeth at different shell lengths in the radiate and non-radiate forms shows that no distinction can be made by using this character. Both show a similar increase in the number of teeth with increasing shell length.



xt-fig. 4. Comparison of mean values of height (*H*) and width (*W*) with length (*L*) for each year group and the range of values for height and width with length. *A*, height/length; *B*, width/length.



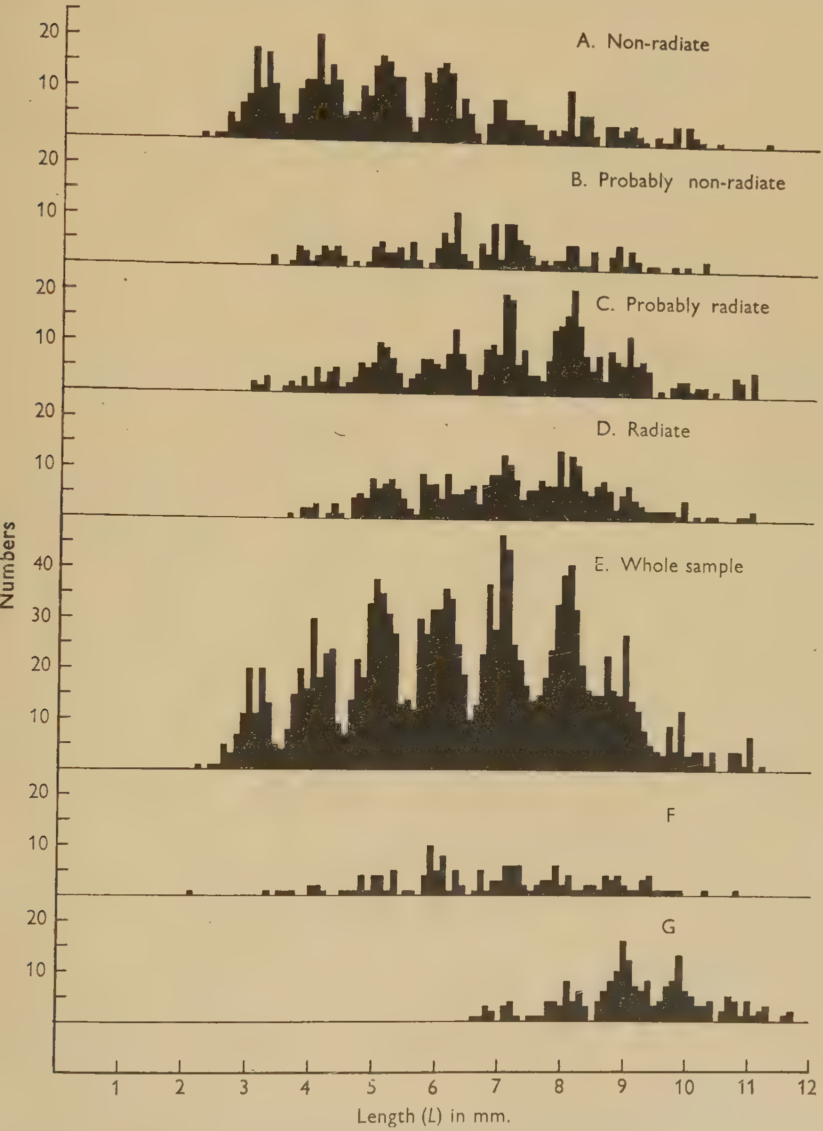
Text-fig. 5. Variation of height with length.



Text-fig. 6. Comparison of the numbers of anterior (A) and posterior (B) teeth at different shell lengths in the radiate and non-radiate forms. ×, radiate; ○, non-radiate; the numerals indicate the number of coincident readings.

AGE AND GROWTH

can be seen that the samples fall into distinct groups (Text-fig. 7); these are esumably year groups though actual proof is lacking. The range in shell gth of each group is identical in the four colour groups examined. The



ext-fig. 7. Population histogram of three samples. A–E, Cumbrae Deep, 31. x. 50.; F, Cumbrae Deep, 17. vi. 51.; G, Minnard Narrows, 27. vii. 51.

undulations of the ranges of variations in Text-fig. 4 also indicate the year groups and again show no difference between the radiate and non-radiate forms. Lebour (1938) reports that the egg diameter of *N. nitida* is 90 μ and that of *N. nucleus*, a species that grows to a similar size, 100 μ. Both species have a very short larval life, but no measurements of the larvae are given. A drawing by Bernard (1896) of a prodissoconch of *N. nucleus* measures 180 μ long, therefore it might be expected that the size of the newly settled spat of *N. nitida* would be of the same order. Lebour states that *N. turgida* from Plymouth spawns in winter and that she successfully reared fertilized eggs to the larval stage in February. If the spawning times at Millport are the same and the growth rate in the first 2 years is of the same order as the following, the smallest group (maximum size 2·7 mm.) of the Cumbrae Deep haul taken at the end of October is probably 2 years old. Although no successful fertilizations were carried out, ripe sperm and ova were present from October to February. The maximum age attained by the Clyde specimens is 10–11 years, but there is a decline in the numbers of each year group after the seventh year. The small numbers in the first two year groups is due to the fact that the mesh size of the net was too great to retain the smaller shells; this also applies to the sample from the Minnard Narrows (Text-fig. 7). The maximum size of the Clyde specimens is less than those obtained by Marshall from the Dogger Bank, the maximum length of which is recorded as from 13·0 to 13·75 mm.

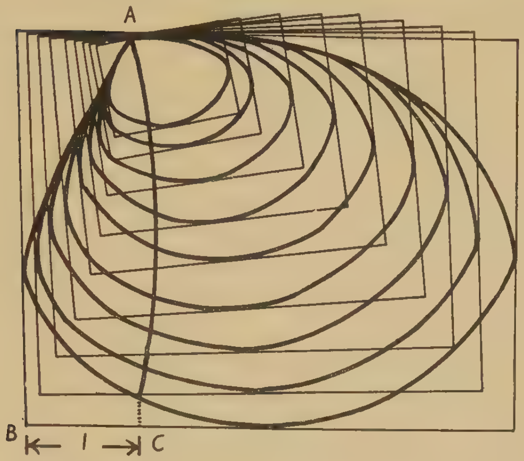
From Text-figs. 4 and 7 it is seen that the shapes of the population curves for each year group in the radiate and the non-radiate forms correspond with each other; this implies that their periods of spawning are the same.

It is obvious that if the shell measurements at various ages in the two groups do not differ significantly from one another then the rates of growth of each are the same. If it is assumed that the cells which lay down the purple-grey pigment remain in a constant position on the mantle edge then the rays indicate the curvature of the growth. Thus the successive yearly increments of growth will be as shown in Text-fig. 8. Until the sixth or seventh year, increments to the shell become greater annually, after this they decrease. Thus the annual increments of height are as follows:

Year groups...	1	2	3	4	5	6	7	8	9
Height	1·90	2·33	3·05	3·82	4·60	5·46	6·35	7·09	7·81
Increments		0·43	0·72	0·77	0·78	0·86	0·89	0·74	0·72

This may be correlated with the fact that the age groups decline in numbers after the seventh year and that this is the peak followed by senescence.

As would be expected, there are variations in the shape of the shells, and the minimum and maximum values from the mean shell values increase with age. From Text-figs. 4 and 5 it has been seen that the minimum and maximum values are of the same order in both the radiate and non-radiate types.



xt-fig. 8. Shell growth calculated from the mean shell measurements of each year group. *AC* is a ray which passes from the umbo to a point on the shell margin where *BC* is the measurement *l*.

DISCUSSION AND CONCLUSIONS

servations have been made on a large number of specimens of the two eged species *N. turgida* and *N. moorei*. In particular, those characters ed by Winckworth as being diagnostic have been studied. These are of ee types: the colour of the shell, the shape of the faeces, and the shape of the ll.

The basic colour is yellow. This may or may not have purple-grey markings. *moorei* is differentiated from *N. turgida* by having these markings in the m of rays. *N. turgida* includes the unmarked form and those shells in which markings are not in the form of rays. It was found that the shells could be anged in a continuous series from a form that was so heavily marked that radiations have merged into one another to give a dark unrayed shell ough forms with varying degrees of radiate markings to those that have ne at all. The shell markings are not necessarily laid down continuously, shells with coloured bands parallel to the lines of growth are found. tudy of a large sample shows that the purple-grey colour does not appear il the end of the second year and that the percentage of unmarked forms reases with age. These facts indicate that there are not two species but one ich exhibits a continuous series of colour varieties. The increase in the nber of specimens which show this coloration as their age increases suggests t the purple-grey colour is a waste product produced by the metabolic vities of the animal. Comfort (1951) points out that 'the shell pigments of lower bivalves are secreted into the shell as a means of disposal, being ived from the diet or unmanageable metabolic residues'.

Examination of the faecal pellets of all the colour varieties gave results that did not agree with those of Moore. Although the types listed by him were found there was no correlation with the type of shell markings. It was mainly on this character that Winckworth (1931) separated the two colour varieties of *N. nitida* into two species. In addition, two other types of faecal pellets were noted.

Examination of many shells (over 2500) shows that the shape is the same in all the colour varieties with no significant difference in any of the shell measurements taken. In addition, there is no difference in growth rate and spawning period. A study of their anatomy does not reveal differences between the colour types.

On the evidence given above it must be concluded that there are no grounds for separating *N. nitida* into two species. As the name *N. nitida* is preoccupied, and as Winckworth has already raised Marshall's variety to specific rank, it is proposed that the name *N. turgida* Marshall 1875 should be applied and *N. moorei* a synonym of it.

I would like to express my thanks to Prof. C. M. Yonge, F.R.S., for his help and encouragement and for reading and criticizing the manuscript. I would also like to thank Dr R. B. Pike for his interest and encouragement and Mr M. V. Brian for his help in the statistics. I am grateful to the Director and Staff of the Marine Station, Millport, for their help and to Mr S. McGonigal for taking the photographs.

This work was made possible in the first place by a grant from the Browne Fund of the Royal Society and later by a Research Grant from the Development Commission.

SUMMARY

In the course of a study of the British Nuculidae the specific characters of a large number of specimens of *N. turgida* and *N. moorei* were re-examined. It was found that no distinction could be made between the two.

Examination of variation in shell colour showed that it was not possible to differentiate into two distinct types. There was a continuous graded series from a dark purple-grey in which the basic yellow colour was almost completely masked, through a series with decreasing numbers of purple-grey radiations, to shells in which the markings could not be differentiated as rays, and finally to shells without purple coloration. The purple-grey colour is not necessarily laid down continuously, and it does not develop until the end of the second year. As the shells get older an increasing percentage of them become coloured.

Although the two types of faecal pellets described by Moore were found they could not be associated with the radiate and non-radiate shell types. The five grooved faeces that he claimed were typical of the radiate variety, were

und in only three of the fifty-six specimens examined. Only one of them as from a definitely radiate shell.

Shell measurements and growth rates were studied, and no feature was und on which two forms could be separated. Samples show that reproducon takes place at the same time in the rayed and unrayed shells.

As the name *N. nitida* Sowerby is preoccupied it is suggested that the name . *turgida* Marshall should now be used with *N. moorei* a synonym of it.

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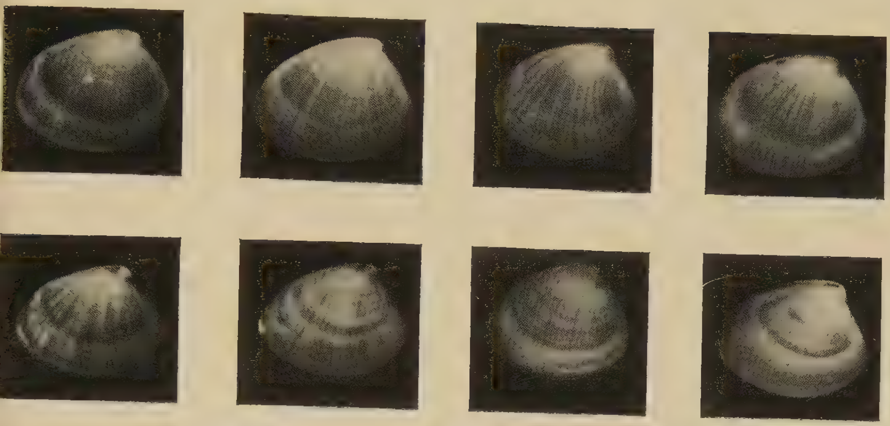
EXPLANATION OF PLATE I

Fig. 1. Series showing the change from a heavily radiate shell to a non-radiate shell. (Some show the sculpturing of the shell, this should not be confused with the purple-grey markings.)

Fig. 2. Left and centre: probably radiate shells; right probably non-radiate shell. These also show shell sculpturing.

Fig. 3. Transverse section of rectum of a radiate shell showing a seven-grooved faecal pellet.



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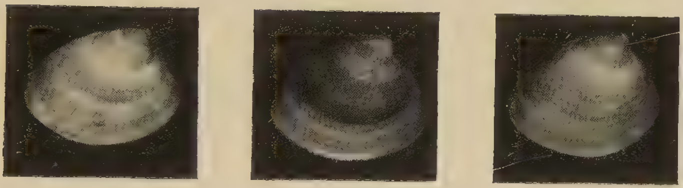

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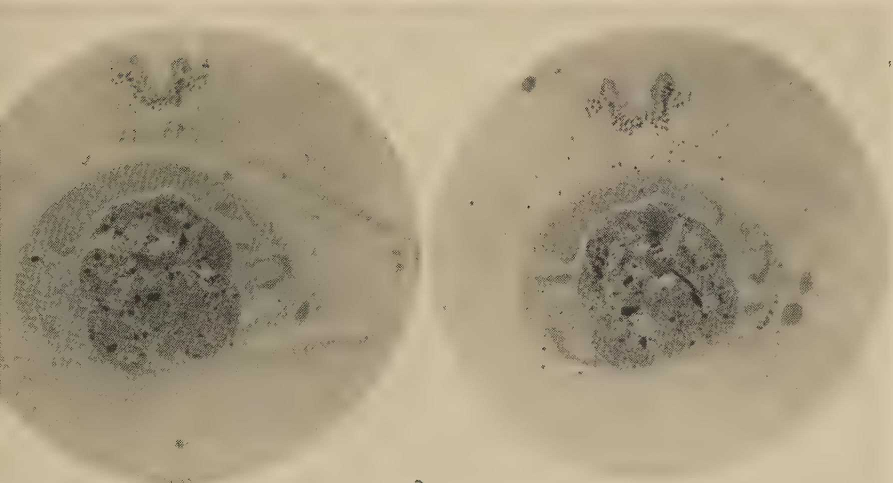

3

OBSERVATIONS ON THE SKIN PIGMENT AND AMOEBOCYTES, AND THE OCCURRENCE OF PHENOLASES IN THE COELOMIC FLUID OF *HOLOTHURIA FORSKALI* DELLE CHIAJE

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(Text-fig. 1)

Black or brown pigments are common among echinoderms and certain of them have been described as melanins—for example by Briot (1906) in unspecified holothurians; Crozier (1915) in *Holothuria captiva* and *H. surinamensis*; Verne (1926) in *H. forskali*; Cornil, Mosinger & Calen (1935*a*) in *H. forskali* and *H. tubulosa;* and Millott (1950) in *Thyone briareus*. The evidence or such statements has not always been made clear, but in certain instances, or example, in *Thyone* (Millott, 1950, and unpublished observations), other holothurians (Briot, 1906), and in the echinoid *Diadema* (Millott & Jacobson, 951; 1952*a*), such pigments have been shown to possess the chemical and histochemical characteristics of melanin. The fact that the black skin pigment f *Holothuria forskali* also shows the characteristics of melanin has been mentioned in a preliminary note (Millott, 1952).

In *Diadema* it was also shown that the pigment arose within the amoebocytes f the coelomic fluid by a process of oxidation involving an enzyme system with the characteristics of phenolases (Millott & Jacobson, 1952*b*).

In view of the frequent association of melanin production with such nzymes, the importance of these observations is obvious, and it is therefore ppropriate to extend them to other echinoderms. This is all the more so, ecause studies on echinoderm pigmentation have been relatively few, and most of these concern echinochrome or allied pigments, and because the rigin of melanin in other forms has proved so difficult to elucidate. Despite xtensive investigation, the process of melanogenesis is still a controversial matter, and there is thus good reason to extend observations on the occurrence f the pigment, and to glean all indications as to how it is produced.

As reported previously (Millott, 1952), *Holothuria forskali* with its abundant lack pigment forms a very suitable object for study, and in this essentially ntroductory and incomplete account evidence will be produced showing that he black skin pigment has the properties of melanin and that, as in *Diadema*, s presence is coupled with the existence of a well-defined phenolase system, distinct from the cytochrome-cytochrome oxidase system. The amoebocytes of the coelomic fluid appear to play a vital part in the formation and distribution of the pigment.

THE PROPERTIES OF THE SKIN PIGMENT

Unfortunately the precise chemical nature of melanin is unknown. Indeed the term would appear to refer to a group of pigments rather than to a specific substance. Specific tests are therefore non-existent, but it is permissible to identify a pigment, provisionally at least, as a melanin, if it has the following characteristics (Lison, 1936): (i) if it occurs in the form of black, brown, or yellow granules; (ii) if it shows extreme resistance to solvents; (iii) if it is decolorized by oxidizing agents; (iv) if it reduces directly ammoniacal solutions of silver nitrate.

As indicated below, the black body wall pigment of *Holothuria forskali* shows these characteristics.

Mode of Occurrence

The body-wall pigment exists in the form of black granules scattered throughout the body wall, sparsely or in irregular patches, which are most numerous in the epidermis and subepidermal connective tissue. The granules are present inside cells as well as in intercellular spaces (see p. 534).

Solubility

The following simple method was used to test the solubility of the pigment.

The densely pigmented superficial layer of the body wall was stripped off and shaken with distilled water. A dark greenish yellow pigment dissolved and the solution was decanted off. The residue was shaken with distilled water and the whole filtered. The precipitate, which formed a dark brown film on the filter-paper, was extracted repeatedly with various solvents. The final filtrate from each solvent was evaporated to dryness on a water-bath to detect small amounts of pigment which might have dissolved. All other operations were carried out at laboratory temperature. The results are shown below.

Solvent	Remarks	Solvent	Remarks
Water	Slightly soluble	Carbon disulphide	Insoluble
Ethanol 90 %	Insoluble	Pyridine	Slightly soluble
Acetone	Insoluble	1·0 N-HCl	Slightly soluble
Benzene	Insoluble	1·0 N-H_2SO_4	Insoluble
Ether	Insoluble	1·0 N-HNO_3	Insoluble
Petrol ether	Insoluble	1·0 N (approx.)-NaOH	Slightly soluble
Chloroform	Insoluble		

Where the pigment proved slightly soluble, the residue obtained by evaporation to dryness was extracted repeatedly with the same solvents. Its behaviour towards all solvents was precisely the same as the original precipitate, thus showing that the latter was not a mixture of similarly coloured substances, nor changed by the action of the solvents.

In general, therefore, the behaviour of the pigment towards solvents resembles that of melanin.

Bleaching by Oxidizing Agents

Small pieces of superficial body wall measuring about 1·5 by 0·5 cm. were subjected to the action of the following oxidizing agents, known to decolorize melanin (Lison, 1936). The results are indicated below:

Agent	Remarks
Chlorine	Pigment bleached completely in 12 hr.
Bromine water	Pigment bleached completely in 12 hr.
Hydrogen peroxide (about 12 vol.)	Black pigment changed to red in 12 hr. Prolonged action of agent led to no further change
2 % chromic acid	Bleached to pale brown in 10 hr.
Potassium permanganate followed by oxalic acid	Bleached completely in 1 hr. after addition of oxalic acid

The pigment thus behaves like a melanin.

The Argentaffine Reaction

Paraffin sections of the body wall, 10 μ thick, were subjected to Masson's technique for the argentaffine reaction (see Lison, 1936), and counterstained n Orange G.

The granular body-wall pigment under study readily reduced ammoniacal silver nitrate.

Thus the black pigment of the body wall of *Holothuria forskali* shows clearly he characteristics of melanin.

THE COELOMIC FLUID

The Amoebocytes

Suspended in the coelomic fluid are numerous amoeboid cells, which in general features resemble those previously described in a wide variety of echinoderms (see Geddes, 1879; Cuénot, 1891; McClendon, 1912; Kindred, 921, 1924, 1926; Dawson, 1933; and Ohuye, 1938).

When examined alive, or after fixation in Bouin, their most striking feature is the large number of cytoplasmic inclusions, certain of which are especially nteresting, taking the form of spheroids (Fig. 1 A and B, *sph.*), which may be olourless, yellow or brown, bearing on their surfaces, deep brown, or black ranules.

The appearance of granules of black pigment in association with cytoplasmic spheroids, at once recalls the condition observed in the amoebocytes f *Diadema*, where it has been shown that melanin appears in association with imilar spheroids (Millott & Jacobson, 1952*b*).

Formation and Darkening of the Coagulum

When removed from the animal and exposed to air, the coelomic fluid slowly forms a sparse white stringy clot, which, after about 12 hr. at laboratory temperatures, breaks up into isolated pale brown globules frequently flecked with black. After a further 12 hr. the entire globule turns black.

Examination by microscope shows that the clot is formed of free and compacted amoebocytes.

In coelomic fluid which has been exposed for several hours, many spheroids (both brown and colourless) lie free in the clot following disorganization of many of the amoebocytes, and it is to the brown spheroids and the associated granules that the colour of the clot is due.

As far as can be judged from appearances, the pigment granules at the surface of the spheroids tend to amalgamate, forming densely brown patches around the spheroids, from which brown colour seems to spread into the spheroid (Fig. 1 A, *b.p.*). This process occurs progressively, and, though the spheroids of many amoebocytes remain colourless, most become brown on continued exposure, until after 2 days the coagulum is seen to consist of a dense network of brown strands formed by the spheroids and debris of disorganized amoebocytes, with isolated, intact and apparently healthy, amoebocytes in the interstices.

Such appearances at once recall the changes seen to occur in the coelomic fluid of *Diadema* (Millott & Jacobson, 1952*b*), where, however, the clot is much more extensive and darkens more rapidly. It is especially significant, however, that here, as in *Diadema*, the darkening involves both spheroids and the granular pigment at their surfaces.

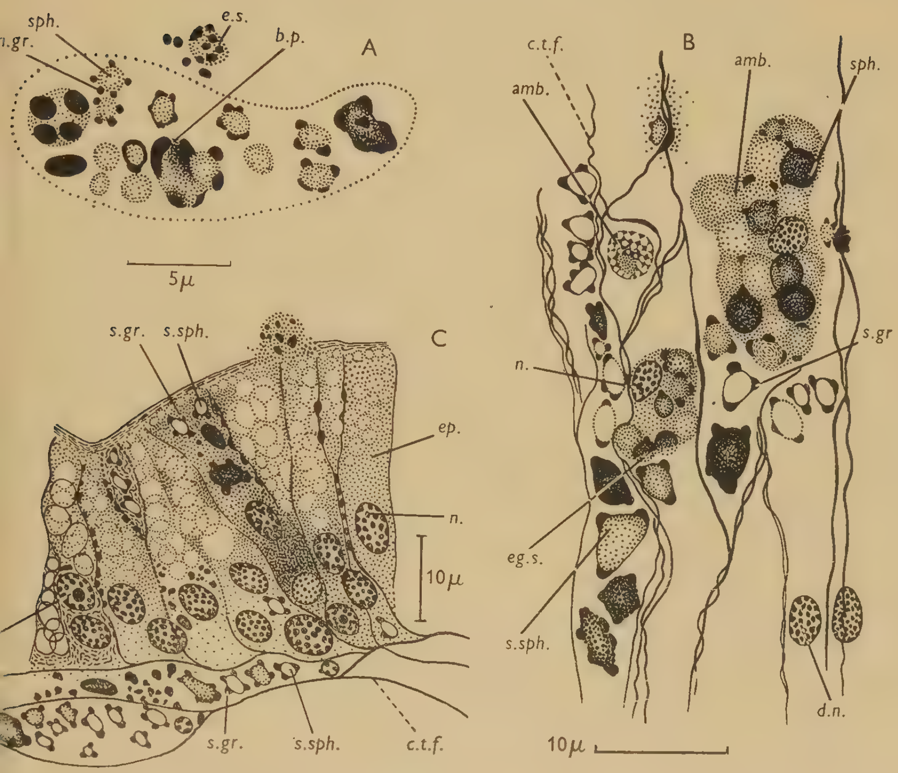
Although it was not possible in the time available to show directly by chemical or histochemical means, as in *Diadema*, that the pigment formed in the amoebocytes is a melanin, that it probably is becomes clear from histological preparations, in which it can be seen that the black pigment of the skin (which as we have already seen has the properties of melanin) is undoubtedly derived from the coelomic amoebocytes (see below).

Such a conclusion is fully supported by the reaction of the amoebocytes to ammoniacal silver solutions, for, after using Masson's technique for the argentaffine reaction, the pigment granules and many of the associated spheroids blackened intensely.

HISTOLOGICAL EVIDENCE OF THE DERIVATION OF SKIN PIGMENT FROM THE AMOEBOCYTES

Methods

Small pieces of body wall were fixed in Bouin's fluid for 24 hr. at laboratory temperature. Sections 10 μ thick were cut in wax and stained in safranin and light green.



ig. 1 A. Living amoebocyte, from the coagulum formed in the coelomic fluid of *Holothuria forskali*, sketched after exposure of coelomic fluid in an open dish for 20 hr. at laboratory temperature and redrawn. B. Portion of the subepithelial zone of the body wall of *H. forskali*, showing amoebocytes and melanin deposits. From a preparation fixed in Bouin's fluid and stained in safranin and light green. C. Part of a section through the superficial region of the body wall of *H. forskali*, showing melanin deposits in epithelial and subepithelial zones. Fixed Bouin's fluid, stained by Masson's method for the argentaffine reaction (see p. 531), counterstained in Orange G.

amb.	amoebocyte.
b.p.	brown pigment spreading into spheroid (see p. 532).
c.t.f.	connective tissue fibre.
d.n.	isolated nucleus (?) from disintegrated amoebocyte.
eg.s.	spheroid apparently being egested by amoebocyte.
ep.	epithelial cell.
e.s.	spheroid eliminated from amoebocyte with granules of pigment.
m.gr.	melanin granule.
n.	nucleus.
s.gr.	melanin granule in body wall.
sph.	spheroid in amoebocyte.
s.sph.	spheroid in body wall (?) eliminated from amoebocyte.

Detailed examination of the deposits of black or brown pigment in the skin shows that the pigment granules (Fig. 1 C, *s.gr.*), in size, form and colour, correspond closely with those in the amoebocytes, and further, that most are associated with bodies which, in range of size and appearance, closely resemble the spheroids (Fig. 1 C, *s.sph.*). This clearly suggests that the skin pigment is derived from the amoebocytes.

Fully in harmony with this are other clear signs of participation of amoebocytes in the deposition of skin pigment, such as the constant presence in all parts of the body wall of numerous amoebocytes indistinguishable from those in the coelomic fluid, especially numerous in the loose connective tissue below the epidermis where pigment is so abundant (Fig. 1 B). Again there are frequent signs of disorganization among the amoebocytes, and occasional indications of spheroids and their associated pigment being cast out from these cells (Fig. 1 B, *eg.s.*) in the body wall.

All histological indications are, therefore, that the pigment is derived from the coelomic amoebocytes, which, wandering into the body wall, unburden themselves of pigment by casting it out or by disintegration. Such a tentative conclusion is fully in agreement with many previous indications of the importance of amoebocytes in the carriage of pigments and metabolites generally, in the bodies of echinoderms (Cuénot, 1891; List, 1897; Kindred, 1926; Lison, 1930; Millott, 1950), and especially with the more revealing results from experiments on *Diadema*, reported previously (Millott & Jacobson, 1952*b*), in which iron saccharate injected into the coelomic fluid was found to be carried by amoebocytes and deposited in the melanophores of the skin.

Again, the histological evidence of breakdown of the amoebocytes in the body wall, with liberation of their contained pigment, parallels the same phenomenon observed to occur in the living amoebocytes of the coagulum (p. 532).

It is interesting to note, in passing, that Cornil *et al.* (1935*b*), working on *Holothuria forskali* and *H. tubulosa*, observed the frequent association of pigment deposits in the body wall with amoebocytes and the appearance of cellular disintegration, and, further, believed that the matter eliminated was re-utilized by the amoebocytes. '...et l'on peut penser que les amas de désintégration sont susceptibles d'être réutilisés par le travail amibocytaire. L'appareil pigmentaire serait ainsi soumis de façon constante à un processus de destruction (pigmentolyse) et de néoformation (pigmentogenèse).'

Finally, the participation of coelomic amoebocytes in melanogenesis is supported by the discovery of a well-defined enzyme system in these cells, which brings about the oxidation of both mono- and poly-phenols, and is distinct from the cytochrome-cytochrome oxidase system. Such enzymes have been shown to be associated with melanogenesis in a wide variety of forms.

The Phenolase System in the Coelomic Fluid

Simple colour reactions, based on those previously employed by Raper (1932) nd Pugh & Raper (1927), were used to reveal the presence of phenolases, oelomic fluid being added to buffered phenolic substrates, oxidation being ndicated by darkening of the solutions. The experimental solutions were made up as follows: 5·0 c.c. coelomic fluid (freshly withdrawn and well shaken to disperse the coagulum) + 5·0 c.c. 0·06 M Sørensen buffer (mostly at pH values anging between 6·5 and 7·0), containing 0·1–0·2 % of the phenolic subtrate + a few drops of toluol or chloroform (as an antiseptic).

In all instances experiments were accompanied by controls in which the oelomic fluid had been boiled and cooled before use. Both experiments and ontrols, frequently inspected and gently agitated, were incubated at 24° C. The controls were especially necessary in view of the tendency of some phenolic substrates to auto-oxidize, and the fact that the coagulum itself darkens on exposure. Usually a little detergent was added both to experiments and controls (see below).

These experiments showed that the coelomic fluid possesses the power to oxidize phenols such as 'dopa', pyrocatechol, L-tyrosine, pyrogallol and cresol mixed isomers) with glycine. The characteristic coloured oxidation products lways appeared in the initially colourless solutions, those containing 'dopa' or tyrosine becoming grey or black, pyrocatechol reddish brown, pyrogallol brown, whilst those containing a mixture of cresols and glycine became scarlet or orange. The controls, with boiled coelomic fluid, showed either no change, or where the substrates tended to auto-oxidize, relatively little change. .

It is noteworthy that detergents, such as 'Cetavlon' (Imperial Chemicals Pharmaceuticals) Ltd.) and saponin, exerted a marked accelerating effect on oxidation. Without detergent, oxidation of the phenolic substrates occurred only slowly, requiring at least 12 hr. at pH 7·0 and 24° C. to bring about distinct colour difference between experiment and control. After adding little detergent pronounced darkening of the experimental solutions as compared with the controls could be observed within 3 hr. at the same temperature, pH, and with the same substrate concentrations.

Thus it is clear that the capacity of the coelomic fluid to oxidize phenols depends on a heat-labile factor with marked surface action. However, it is not yet clear whether this is an enzyme system or not, since such oxidations can be brought about by heat-labile factors such as copper-protein complexes that are not usually regarded as enzymes (see Bhagvat & Richter, 938).

Two features of the reactions, however, namely their sensitivity to potassium cyanide and pH, indicate the participation of enzymes. Thus oxidation of L-tyrosine and pyrocatechol at pH 7·3 is strongly inhibited by concentrations of potassium cyanide as low as 0·0002 M, and the oxidation of tyrosine occurs most readily in solutions buffered within the pH range 6·0–7·5 at 24° C., falling off rapidly on either side.

Owing to the limitations of the method employed, it was pointless to attempt the determination of a precise optimum; nevertheless it is clear that the range of activity corresponds with that of tyrosinase (Raper, 1928).

It now remains to be discovered whether the enzyme system indicated is to be found in the coelomic fluid, or in the suspended amoeboid cells. That it is present only in the cells can be shown by separating them by centrifuge, boiling the remaining fluid, and after cooling, reconstituting the coelomic fluid by replacing them in the fluid. The reconstituted fluid thus formed shows an undiminished power to oxidize phenolic substrates, and thus the heat-labile factor I believe to be an enzyme system, must be present in the amoebocytes, and not in the fluid.

Such experiments have a further significance in showing that there is no heat-labile factor in the fluid inhibiting oxidation, as was found in *Diadema* (Millott & Jacobson, 1951), since no increased oxidizing power resulted from the treatment.

The existence of an enzyme system in the amoebocytes capable of oxidizing phenolic substrates at once leads us to suspect as responsible, in some measure at least, the widely distributed cytochrome-cytochrome oxidase system. However, the fact that mono-phenols such as L-tyrosine are oxidized shows that this system cannot be entirely responsible. The sensitivity of the cytochrome-cytochrome oxidase system to sodium azide at pH 7·3, and to acetone, shown by Keilin (1936) and Keilin & Hartree (1938), enables us to eliminate this system as responsible in any significant measure for the effects described here, for the oxidation of polyphenols such as catechol was not affected either by 0·002 M sodium azide at pH 7·3, or by acetone.

There are thus clear indications that the coelomic amoebocytes of *Holothuria forskali* contain an enzyme system of the phenolase type commonly associated with melanogenesis.

It is now appropriate to point out certain peculiar features in the system. First, thiourea exerted but slight inhibitory effect, at a concentration of 0·001 M, on the oxidation of tyrosine at pH 6·5 and 24° C.; and no discernible effect, at a concentration of 0·0005 M, on the oxidation of pyrocatechol at pH 7·3. Phenolases are characteristically sensitive to substances binding copper ions, so that this is an unexpected property of the system. Secondly, in some preliminary experiments, it was found that irradiation with ultra-violet light exerted but a small and inconstant effect: 5 min. irradiation of coelomic fluid by means of a 'Hanover' mercury arc, sometimes increased (but only slightly) its power to oxidize L-tyrosine at pH 6·5. Irradiation for longer periods diminished it. This too was unexpected, as similar treatment of the coelomic fluid of *Diadema* was found to produce the normal effect and activate the enzyme system (Millott & Jacobson, 1952*b*). These indings are advanced with due caution, however, as it is considered that more experiments are necessary, especially to eliminate possible heating ffects.

DISCUSSION

The foregoing investigation, though regrettably incomplete, is adequate to confirm previous indications of the occurrence of melanin in *Holothuria orskali*, and to show that the occurrence of melanin in this form is coupled with the presence of an active phenolase system. The association is noteworthy in view of many indications of the participation of such enzymes in melanogenesis in a wide variety of animal forms, but especially because of heir importance in the echinoid *Diadema*. Thus in both echinoderms the nzyme system occurs in the histologically similar coelomic amoebocytes, which in both produce a black pigment with the characteristics of melanin. Further, it is most significant that the pigment appears in the amoebocytes of both, in association with cytoplasmic elements, the spheroids.

The association of granules of melanin with spheroids is remarkably onstant, not only within the amoebocytes, but also in the skin (Fig. 1 c), and he ubiquity of the association suggests that the spheroids are involved in igment formation, a suggestion which is fully supported by the histological vidence brought forward on p. 534. This is most significant, for in *Diadema*, where the same association prevails, there is evidence that the spheroids and heir immediate surroundings are regions of high redox potential, a factor nown to be important in melanogenesis (Figge, 1940; Dawes, 1941; Schuppli, 950).

The enzymic activity of the coagulum formed in the coelomic fluid, in its apacity to bring about oxidation of both mono- and poly-phenols, sensitivity o cyanide, pH range and insensitivity to sodium azide and acetone, resembles hat of tyrosinase, an enzyme that has been widely associated with melano-enesis. In the absence of more precise experiments, it is clearly unprofitable o speculate as to whether one or more enzymes are involved, a matter that as proved difficult to decide even with refined techniques (see Dennell, 947; Lison, 1936; Greenstein, 1948; Lerner & Fitzpatrick, 1950; Schaaf, 950; Kertész, 1951). It is for this reason that I have at all times preferred to efer to an 'enzyme system' rather than to use more committal terms.

The indications of the participation of cytoplasmic elements such as the pheroids in melanogenesis are interesting in relation to the controversy as o whether melanin is of nuclear or cytoplasmic origin (see Verne, 1926; Meirowsky, Freeman & Fischer, 1950; Meirowsky & Freeman, 1951; etc.), ut it should be borne in mind that the relevant evidence cited in this vestigation is largely histological, and can be advanced only as an indication, nd the possible origin of some of the melanin at least, from the nucleus, has een by no means excluded. Clearly further work is required, but it may be noted in passing, that it is doubtful whether the distinction is worthy of much emphasis in the light of modern conceptions of cellular metabolism.

Finally, it must be emphasized that the incomplete information obtained makes it unprofitable to attempt to formulate a conception of the process of melanogenesis in *Holothuria*, as was done with *Diadema* (Millott & Jacobson, 1952*b*), but it must be admitted that, so far, the processes seem remarkably similar, and further comparison must necessarily await further work.

My thanks are due to Dr H. G. Vevers, Dr F. W. Jacobson, and to Prof. E. Meirowsky for help in connexion with references, and to the Director and staff of the Plymouth Laboratory of the Marine Biological Association of the United Kingdom, who kindly gave me facilities to work during a short but pleasant visit.

SUMMARY

The black body-wall pigment of *Holothuria forskali* shows the characteristics of melanin.

From histological evidence it appears that the pigment is formed in association with the amoebocytes of the coelomic fluid, which eliminate the pigment in the body wall.

The amoebocytes contain a phenolase system, distinct from the cytochrome-cytochrome oxidase system, with the properties of tyrosinase.

The relation of these findings to those of a preceding and more complete investigation into melanogenesis in *Diadema* is discussed.

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PONTANEOUS SQUIRTING OF AN ASCIDIAN, *PHALLUSIA MAMMILLATA* CUVIER

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(Text-figs. 1–16)

Introduction

he simple monascidian *Phallusia mammillata* Cuvier was found by the ithor to exhibit rhythmical spontaneous activity during long periods of oservation in the laboratory (Hoyle, 1952) The movements consist principally quick contractions of the whole body-wall together with water ejection om the branchial and atrial cavities. The water movements are of the nature squirts similar to those which the animals give in response to a violent ternal stimulus, and are accompanied by siphon-rim closure as an inevitable nsequence of the contraction of the body-wall musculature. The closures n be recorded without disturbing the animals unduly by a method which was scribed in the same paper. A slight inward movement of the test can be oserved as the total volume of the animals diminishes during the squirts. The esent work is a study of the frequency patterns of the spontaneous squirting *Phallusia*, together with a brief study of the physiological mechanisms volved in its production and a consideration of the possible functional gnificance of the activity.

The phenomenon of spontaneous activity in ascidians has not attracted uch attention. Polimanti (1911) observed spontaneous opening and closing the siphons of *Ciona intestinalis* at high temperatures (30° C.) but does not scribe spontaneous movements under more normal conditions. Several rlier workers on ascidians observed activity only in relation to the definite ection of foreign particles, faeces or sexual products (Magnus, 1902; öhlich, 1903; Jordan, 1908). The phenomenon was first clearly described Hecht (1918) working with *Ascidia atra* from the Bermuda coast. He found ontaneous contractions of the branchial siphon at intervals of about 5 min. unspecified temperatures. He observed contractions visually in a colonial ecies *Ecteinascidia turbinata*. In considering the functions of the rhythm echt examined the possibilities that the movements may serve either to nove waste or to subserve respiration. He rejected both possibilities by gument based on his earlier observations on the ciliary current (Hecht, 1916). finally suggested that the rhythm may be 'the degenerate remains of an ivity homologous with the rhythmic pulsations of the salps', and that 'no action can be ascribed to this rhythmic occurrence'. This suggestion begs the question of phylogenetic relationships within the Tunicata and assumes the salp activity to be a primitive locomotory mechanism. The work of Hecht was followed by that of Day (1919), who observed spontaneous contractions in amputated branchial siphons of *Ciona*. This observation indicated that the nerve ganglion is not necessarily responsible for the rhythmicity, but Day did not observe spontaneous activity in the intact animal.

The problem of the nature of the activity and its relation to the ganglion was more completely investigated by Yamaguchi (1931), who showed that in *Styela clava* the rhythmicity affects both siphons synchronously. Marked contractions occur at intervals of about 8 min. in running sea water, together with smaller contractions at irregular intervals (published record). The rhythmicity became much more marked and also more frequent after a period in filtered sea water. The interval was then reduced to about 3 min. After ganglion extirpation the siphons still showed spontaneous activity, but the co-ordination was lost and the frequency was very variable. Moreover, the frequency was now markedly increased and the amplitude reduced. Yamaguchi also recorded spontaneous activity from both amputated oral and atrial siphons. According to Yamaguchi the normal spontaneous contractions of *Styela* are of two kinds. One concerns the circular muscles and results in water expulsion. The other consists of logitudinal muscle contractions and siphon closure which does not involve water expulsion. The latter is less frequent. Yamaguchi does not give measurements of the relative frequencies, but from his published records the ratio seems to be about 1 : 6. In his paper he makes no attempt to explain the functional significance of the rhythm.

Bacq (1935), in commenting on the spontaneous activity of *Ciona*, remarked that after ganglion extirpation he found the spontaneous activity more frequent and of reduced amplitude. Ganglion extirpation results in reduced muscle tone. Miss P. Kott has recorded spontaneous activity in *Ciona intestinalis*, *Ascidia aspersa* and *Molgula hattensis*, but unfortunately she has discontinued this unpublished work (see Report of the Council of the Marine Biological Association in *Journ. Mar. Biol. Assoc.*, Vol. 30, 1951). The most recent published comment on the activity was that of Young (1950), who suggested that the automaticity may be something in the nature of a hunger contraction.

PATTERNS OF SPONTANEOUS ACTIVITY

In Intact Animals

About sixty normal *Phallusia* have been observed altogether at either the spring or autumn of two seasons. All these animals showed spontaneous activity. With only two exceptions the contractions of both siphons were synchronous except for small movements caused by vibrational and other stimuli. Most of these animals were kept in tanks of the Plymouth aquarium under circulation for a few hours before observation. Some, however, were

bserved immediately after collection some 5 hr. previously. In the latter
nimals the siphons opened slowly, taking about 4 hr. to open completely.
pontaneous movement is apparent after the siphons begin to open in all
stances and the contractions increase in amplitude as the siphons open more
idely. The frequency of the contractions of these animals is high at first and
radually reduces in Plymouth tank sea water. Eventually a nearly constant
hythm of contraction is apparent, with spontaneous squirts at 6–9-min.
tervals. All the observations were at temperatures from 11 to 14° C.
lthough this type of activity is most commonly recorded it changes at times
other equally regular patterns. These patterns include one in which large
ntractions alternate with a number of smaller ones (Fig. 10, lower record).
ome of Yamaguchi's animals showed this pattern, and he described the large
ntractions as due to longitudinal muscles and the smaller ones to circular
uscles. It is not possible to distinguish muscle types clearly in *Phallusia*,
here the general muscle pattern appears to be very irregular. This type of
tivity is not common in *Phallusia*. Another type consists of bursts of squirts
ternating with periods of rest. This type of variation recalls the familiar
atterns of *Arenicola* preparations (e.g. Wells, 1949).

By far the commonest variations are simple increases or decreases of
equency. The changes to a different frequency occur fairly abruptly, some-
mes very abruptly, and are often prefaced by a short burst of vigorous activity
ig. 1 D). The new rate is almost exactly twice or one half the original one and
always maintained for a few hours. It is possible to distinguish slow,
edium and fast rates of spontaneous squirting (Fig. 1, A–C). The animals
ere kept in 20–30 l. of sea water which was changed usually once a day. The
lue of intervals between squirts averaged approximately 16 : 8 : 4 min. The
te changes were not accompanied by any change in the amplitude of con-
action. Sometimes, after a fresh supply of outside sea water, an animal will
ange to the slow rate after about 2–3 hr., maintain this for a further 4 hr.,
en transfer to an ultra-slow rate (intervals of about half an hour) for about
r., during which time the amplitude gradually declines until the spontaneous
ntractions are only just perceptible. The first animal showing this behaviour
as discarded in the belief that it was dying, but when another followed suit
is was left overnight and was later found to have started again at the medium
e. Later, after a new supply of the same outside sea water, it repeated the
rformance. When the amplitude decreased, the branchial siphon declined
st, the atrial later. When the amplitude increased again the branchial siphon
creased before the atrial. The observations show that the decline in fre-
ency is unlikely to be due to poor condition of the animals.

In Operated Animals

Since the squirting is rhythmical it is reasonable to suppose that there must
a pacemaker controlling the frequency. This may be situated in the

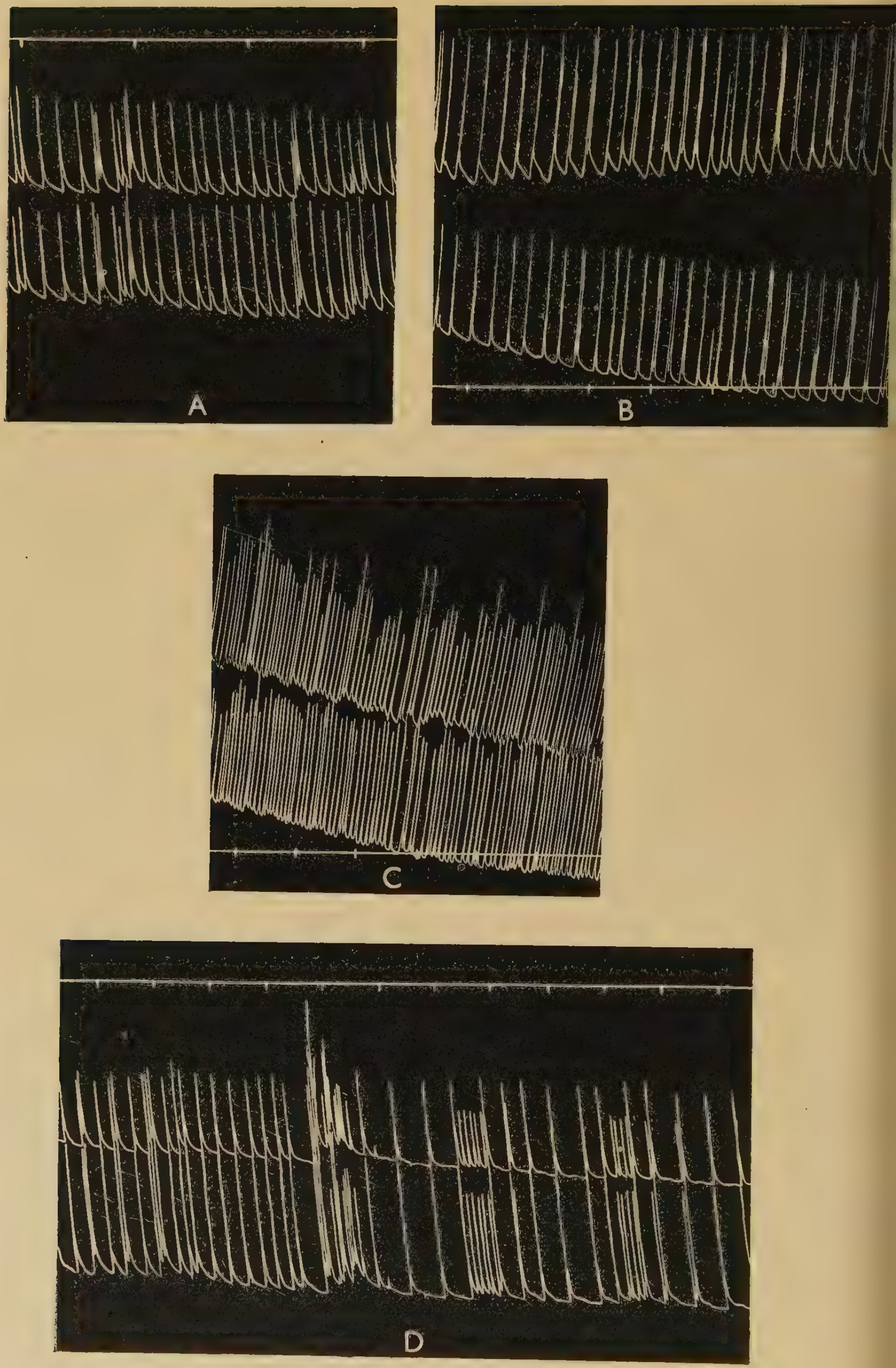
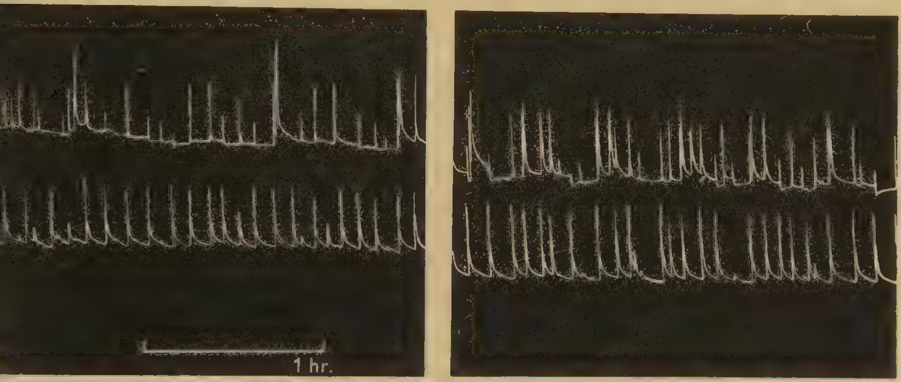


Fig. 1. A, the ‘normal’ 8 min. rhythm; B, the ‘slow’ 16 min. rhythm; C, the ‘fast’ 4 min. rhythm; D, spontaneous change-over from the ‘medium’ to the ‘slow’. Time marks A–C, 1 hr.; D, 30 min. Upper record branchial siphon, lower record atrial siphon.

anglion, in one or other of the siphons, or in some part of the body-wall. The purpose of the experiments described under this heading was to attempt o determine the site of the hypothetical pacemaker. The first experiments consisted of recording from the siphons of deganglionated animals. The nethod of operation to remove the ganglion has been described by Carlisle 1951). Animals in which the ganglion has been removed recover from the operation in a few hours and again show spontaneous activity. This is very variable from one animal to another. The amplitude of the contractions is nvariably reduced. The atrial siphon tends to maintain the medium rate vhilst the branchial siphon, although sometimes contracting at the same time as the atrial, also shows many other erratic contractions (Fig. 2). The activity s relatively constant over many hours regardless of the state of the bathing



ig. 2. Movements of the siphons of a deganglionated animal. Upper record branchial siphon, lower record atrial siphon. Filtered sea water. An interval of 10 hr. separated the two tracings. Note the occasional positive openings of the branchial siphon.

ea water and food supply, and the spontaneous changes of pattern seen in ntact animals are not apparent. In other experiments the ganglion was left ndamaged, but the nerves leaving it and passing to a siphon were cut close o their point of attachment to the ganglion. After this operation there is a eneral loss of tone as evidenced by the more sluggish responses of both the iphon-rims. There is a tendency at intervals for the denervated siphon to give continuous series of closely spaced contractions. These summate to close hat siphon and expel water from it almost completely.

Whole siphons were amputated, and after about 6 hr. records were made of he activity of both the amputated siphons and the intact ones. No movements f amputated siphons occurred. The intact siphons, after remaining closed for few hours following the operation gradually open. When fully open the ntact siphons, both atrial and branchial, resume a fairly normal rhythmicity ut with considerable amplitude variations (Fig. 3). The results are in general greement with those of Yamaguchi (1931), but whilst both he and Day (1919) observed or recorded spontaneous movements in amputated pieces of siphon, no such movements are observed with *Phallusia* material. In this animal there is a strong body-wall attachment only at the siphon rims and contractions of the body-wall in the cut portion could not affect the siphon rim very much even if they were vigorous.

The general conclusion from all these results is that the pacemaker is not situated in either the ganglion or a particular siphon. So far only body-wall muscle from the intersiphonal region has been examined for the presence of purely local spontaneous activity. A strip of muscle 3 cm. long can easily be obtained from the intersiphonal region of the left side. Such strips have been dissected out and mounted vertically with the free end attached to an isotonic lever. These strips show spontaneous slow contractions on which quicker movements are superimposed. The movements of one of these strips were recorded at the same time as those of the siphons of a deganglionated animal and are illustrated in Fig. 7. The quick movements are irregular, but occur at similar intervals to the movements of the branchial siphon of the deganglionated animal. It is just possible that the isolated body-wall strip movements are myogenic: all parts of the body-wall may have a similar

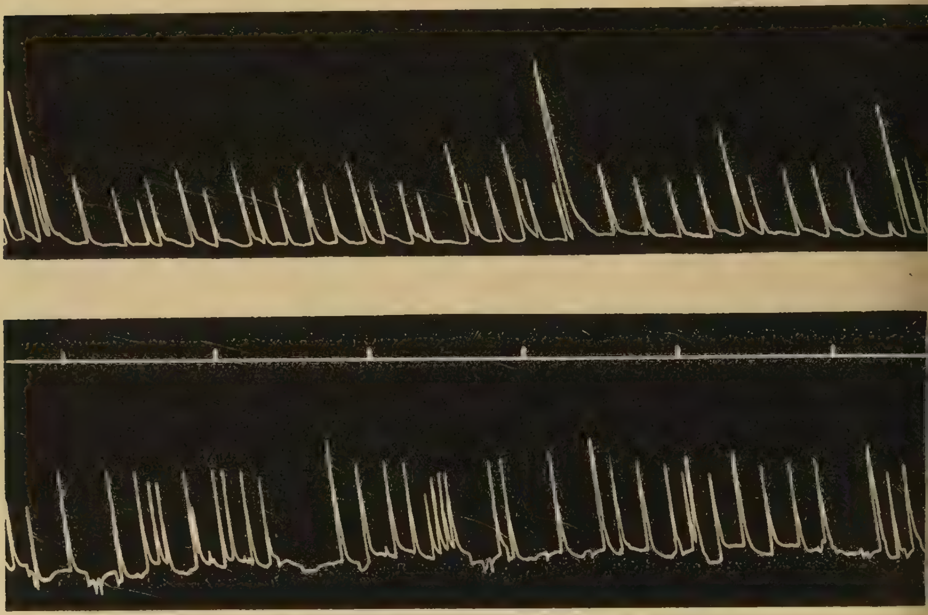
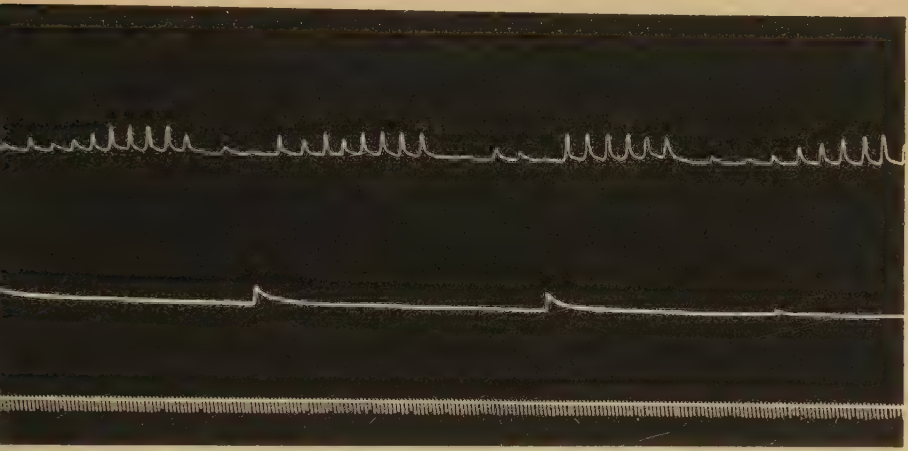


Fig. 3. Upper tracing from branchial siphon after amputation of the atrial. Lower tracing from an atrial siphon after amputation of the branchial siphon. Note the occasional positive openings of the atrial siphon. Time in hr.

ctivity, but this remains to be proved. Also, since Hunter (1898) has figured ell-bodies in nerve-muscle strips of an ascidian, and since the strips used ndoubtedly included some fine nerve elements, the possibility must be in- luded that diffuse nerve elements act as local pacemakers. However, the bservations suggest that rhythmicity is a natural property of the body-wall. n the whole animal this is subject to considerable co-ordination and control y the ganglion, but there is still some co-operation even in the absence f the ganglion, especially within a siphon.



ig. 4. Peculiar pattern of activity shown by one specimen in which the nerves to the atrial siphon had been cut. The amplitudes were quite small. Upper record branchial, lower record atrial. Time in min.

In fatigued and operated animals the spontaneity often presents peculiar atterns, but these always suggest that the basic phenomena are cyclical. One ich pattern is shown in Fig. 4. The nerves to the atrial siphon had been cut, id the pattern illustrated soon developed and was maintained for several ours. The amplitude of the branchial siphon fluctuated in a periodic manner id the atrial siphon contracted at times corresponding only with the minima f the branchial siphon movements. Periodic fluctuation of the branchial phon amplitudes is not uncommon in intact animals. The interval between ie modulation peaks is then about 45 min. The mechanical response of a phon to electrical stimulation is partially a function of the previous history f the animal, partly a function of the stage in the spontaneous activity cycle Ioyle, 1952). A single excitation given just before a spontaneous squirt is ie frequently elicits a large response. A single excitation given just after a ontaneous contraction tends to set up a series of closely spaced contractions. oth phenomena are present in operated animals. Thus, in Fig. 5, a single shock produced the latter effect although the nerves to the atrial siphon had been cut. After a short period of electrical stimulation the frequency of spontaneous squirting is reduced. The previous rate is regained fairly quickly (Fig. 6). All these phenomena must be due to some effects of external stimuli on the basic mechanisms which determine pacemaking in the body-wall.

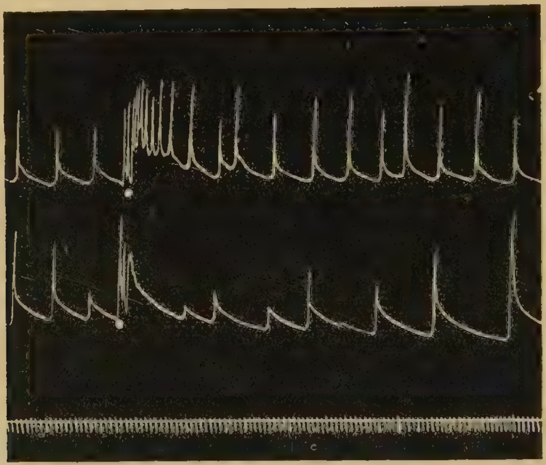


Fig. 5. The consecutive series of contractions in response to a single electrical stimulus given immediately after a spontaneous squirt evidenced in an animal with the nerves to the atrial siphon (lower trace) severed. Time in min.

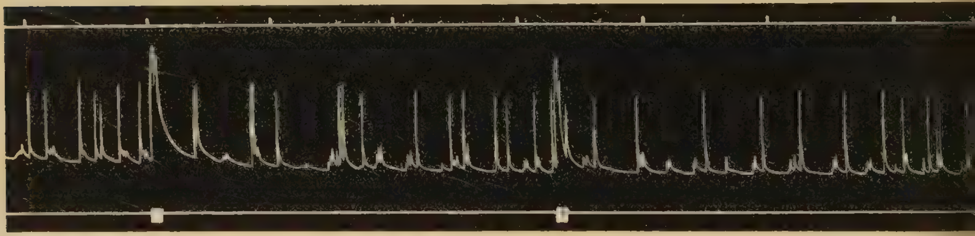
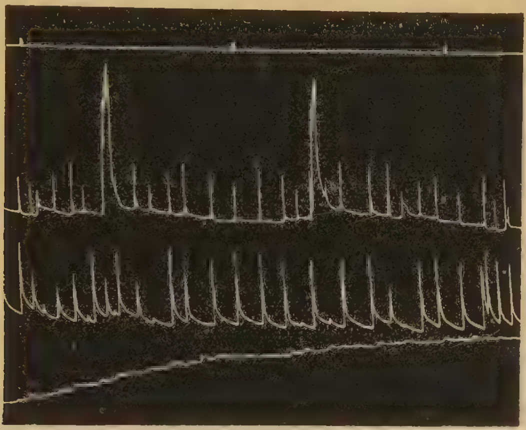


Fig. 6. The effect of a series of electrical impulses at 1 in 2 sec. intervals (indicated on the lower line) on the frequency of the spontaneous activity. Branchial siphon. Time in hr.

VOLUMES OF SEA WATER DISPLACED

The classical interpretation of feeding in sedentary monascidians is that they extract food particles from a continuous stream of water. This stream is maintained by the activity of cilia situated on the inside of ostia which perforate the branchial sac. The stream enters the branchial siphon, passes through the perforations and leaves via the atrial siphon (Fol, 1876; Roule, 1884; Orton, 1913). This current is supposed to be continuous, although no long-term bservations have been made. Squirting, on the other hand, produces no low through the pharynx; the water is expelled from the whole branchial and trial sacs through the respective siphons at the same moment. It is of coniderable value to know the actual volumes displaced by the two activities and o compare them. The only published observation on the rate of the throughurrent with which I am familiar, and one which is often quoted, is by Hecht 1916). Hecht fitted a glass tube into the atrial siphon of *Ascidia aspersa* and hen fed the animal on carmine particles. When the particles appeared in the xhalent stream he measured the time taken for them to traverse the length of he tube. By a simple calculation (not specified), he deduced that a 100 g. nimal passes 173 l. of sea water per day. This means an average output of



ig. 7. Spontaneous movements of an isolated strip of body-wall from the intersiphonal region. The movements are the lowest tracing and are recorded at the same time as those of the siphons of a deganglionated animal. Upper record branchial, lower atrial. Time in hr.

c.c./sec. Hecht's observations are open to some criticism. He appears to ave multiplied the rate of movement of particles along the tube by its cross-ectional area in order to obtain rate of flow values. This assumes the rate to e uniform across the tube. In practice it is a function of the distance from e walls. There is no flow in the region in immediate contact with the walls. he rate increases towards the centre, where it is maximal, by a factor which is roportional to the square of the distance, i.e. the curve relating rate of flow distance from the wall is parabolic. The Poiséille formula for the quantity liquid, Q, flowing through a tube in unit time is

$$Q=\frac{\pi}{8}\frac{pa^4}{\eta},$$

here p = pressure drop per unit length of tube, η = viscosity of liquid, and

a = diameter of tube. When the formula is derived assuming the velocity to be uniform it becomes

$$Q=\frac{\pi}{4}\frac{pa^4}{\eta}.$$

That is, Hecht's values are probably too large by a factor of × 2, since most of the particles will travel down the centre of the tube if the conditions for smooth flow are satisfied. In practice also, Hecht's higher rates are such that they give Reynold's numbers of the order of 2000. A Reynold's number of 1000 or greater is taken as an indication of the conditions for turbulent flow, which would make measurements by Hecht's method impossible. As a further check on the possible validity of these high figures, I have arranged a constant-flow device to deliver sea water containing carmine particles through a tube with the same dimensions as Hecht's and find difficulty in following the progress of any particles through the tube when the rate of flow is 2 c.c./sec.

The ciliary current of *Phallusia* has been measured in two different ways.

(1) The first method gave an intermittent record. A cannula with a wide tube and long, right-angled, narrow-bore side arm was inserted in the branchial siphon. The cannula and side arm were fixed in a horizontal position. At 2 min. following a spontaneous squirt, when the animal was quite undisturbed, the main arm was suddenly closed. The rate of water uptake was then measured by timing the movements of an aniline bubble along the side arm. With this method the greatest rate of flow observed was 4 c.c. per minute in Plymouth tank sea water at 14° C., during March 1952. The flow was not infrequently zero in the same individual. A rough average figure was 1 c.c./min.

(2) The second method was devised to give a continuous record of the rate of flow. It was similar to that used by Wells & Dales (1951) for recording the circulation of water in the tubes of Polychaete worms. A special cannula was gently inserted into an open atrial siphon. The bulb of the cannula was made just larger than the diameter of the siphon. The natural seal thus formed was capable of withstanding about 12 cm. pressure of sea water. The cannula was mounted vertically with its side arm horizontal. The wide vertical tube contained a float which was attached to a lever writing on a kymograph (Fig. 8). The side arm opened to the exterior through a short length of capillary tubing which could be by-passed for null-level determinations by a T-piece with pinchcock. The whole was covered with sea water (about 20 l.) to a level above the side arm. The device could be calibrated by calculation (see Wells & Dales), given the magnitude of the capillary, lever amplification, etc., or a constant-flow device could be inserted in place of the animal. The ciliary flow maintains a continuous depression below the null-level line, whilst the squirts are also recorded quantitatively as superimposed spikes. A typical atrial siphon record obtained by this method is shown in Fig. 10. The contractions appear slightly less regular in frequency than those recorded by the

siphon-rim closure method. Hence it is probable that the cannula constitutes a greater disturbance than the lever arms. Also, the method interferes to some extent with defaecation. However, one animal survived a week of this treatment without obvious deterioration. The method can also be used to determine branchial siphon activity by using a larger cannula and either removing or increasing the diameter of the capillary input resistance so that free access to the sea water is possible. The ciliary through-current is not then recorded.

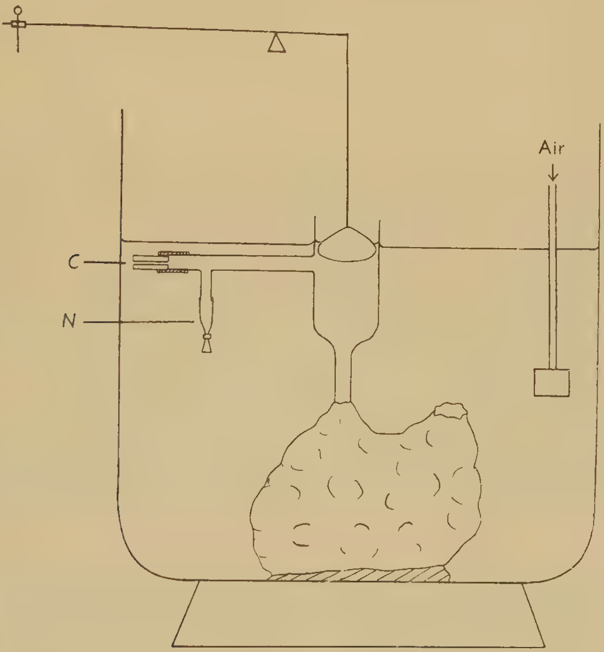


Fig. 8. Method of recording continuous flow and squirting simultaneously. *C*: capillary resistance; *N*: null-level indication pinchcock.

With the second method of recording it is seen that the branchial sac squirts bout 12 c.c. and the atrial about 10 c.c. The average capacity of the branchial ac of a full-sized *Phallusia* is 17 c.c. The corresponding atrial sac holds about 4 c.c. These are necessarily rough values owing to the difficulties involved in naking the determinations, but it is apparent that each sac expels some two-hirds of its contents on each squirt. A tracing from a fast record of a branchial phon squirt is shown in Fig. 9. This was obtained by the float method. The ater ejection is very rapid, occupying only $\frac{3}{5}$ sec. With the float method the ontinuous depression due to the ciliary current again shows it to have an verage value of only 1 c.c./min. This represents a pressure in the side arm f 2 mm. water, which may be as large as the current is capable of producing.

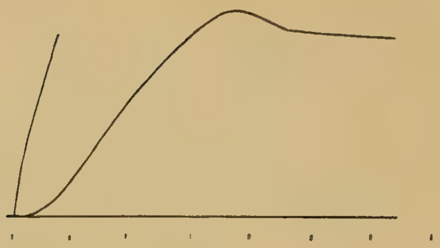
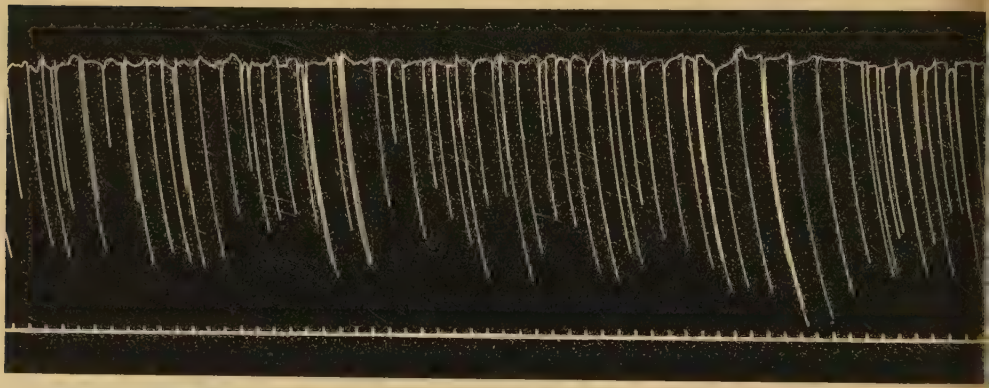


Fig. 9. Tracing from a fast record of a spontaneous squirt. The first stroke marks a calibration injection of 10 ml. with the drum stationary. Time in $\frac{1}{5}$th sec.



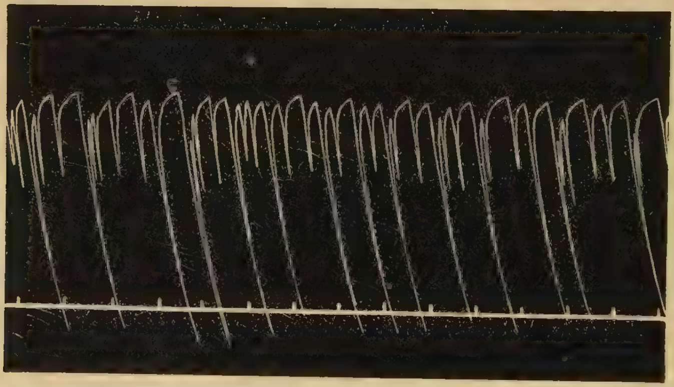


Fig. 10. Upper record: typical record of atrial siphon activity by the flow method. Time in 10 min. Lower record: variant of above showing alternate large and small contractions. Smaller diameter capillary than in upper record. Time intervals: 10 min.

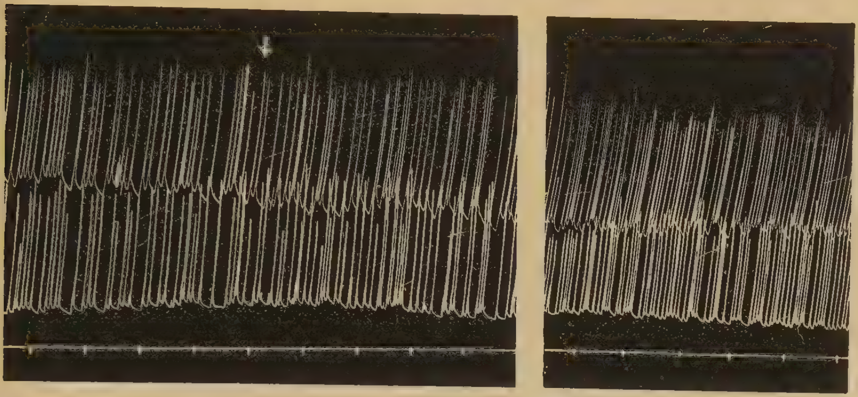
hus one might suspect that the real value might be larger than 1 c.c. Howver, the value was often less than 1 c.c./min., as recorded with the same ethod. We are therefore left with the problem of the still enormous difference etween the *Phallusia* values for the through-current and the Hecht values hich, even when allowance for possible errors in method are made, show the iliary current to be some 100 times greater. Further, we must consider hether the observed *Phallusia* values would be adequate to subserve espiratory and nutritional requirements.

On the first of these points, an observation of considerable importance is hat when carmine or graphite particles are fed to *Phallusia* they do not appear n the exhalant current. They are not again apparent until they are ejected as aeces. If very thick suspensions are added some of the particles are ejected rom the branchial siphon at the next squirt. However, it is clear that the nimals are very efficient at removing particles from the water going in between quirts. If Hecht's carmine particles were coming out of the atrial siphon lmost immediately, how were they getting through the mucus film lining the ranchial sac so quickly? Why were they not picked up by the feeding echanism? Is this mechanism capable of selecting food particles and rejecting armine? Some ascidians are known to have large slits connecting the anterior art of the pharyngeal sac directly with the atrial cavity. These slits were first escribed by von Kupffer (1875) and were called pharyngo-cloacal slits by arstang (1891). Their function is uncertain, although Garstang offers the uggestion that they may serve to allow water from the contracting branchial iphon to flush through the atrial siphon and so help to remove accumulated aeces. I have observed carmine particles fed to *Ciona intestinalis* passing hrough such slits and passing directly out of the atrial siphon a few seconds fter entering the branchial siphon. Clearly, the behaviour of these particles annot indicate the fate of particles within the main body of the branchial sac hich can presumably trap and remove them efficiently. Further, in view of he ease with which a contractile response can be elicited, it is just possible hat Hecht's observations were unwittingly made on particles passing through hese slits and subsequently being forcibly ejected by actual contraction of he body-wall.

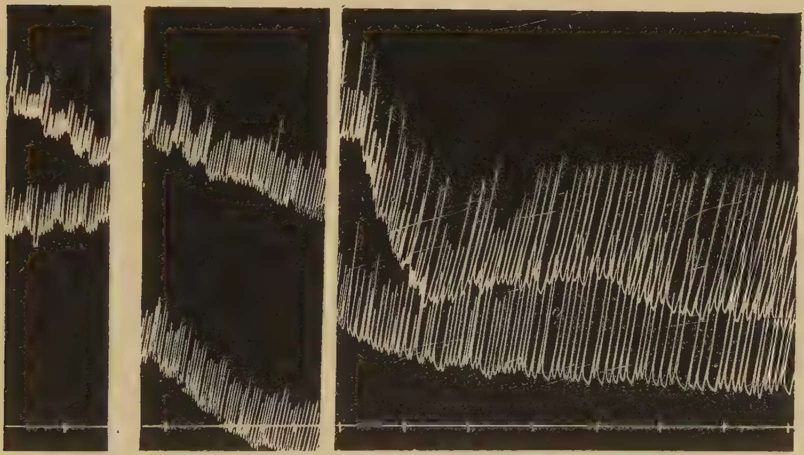
The second point to be considered is the functional adequacy of the low alues observed in the *Phallusia* experiments. Observations on the nutritional equirements of ascidians were made by Moore, Edie, Whitley & Dakin (1912). hey found that a group of small unidentified ascidians of capacity 10 c.c. ould be expected to obtain adequate oxygen and more than adequate food, n the assumption that metabolism is constant, from 150 c.c. Port Erin outside ea water per hour. If we assume a *Phallusia* to require 2–3 times this volume hen the observed through-current is clearly inadequate. The water brought n by the current is not, however, the only source of food and respiratory gas. espiration is also served by both the branchial and the atrial pumping. The former drives about 90 c.c./hr. and the latter about 76 c.c./hr. into contact with the internal body surfaces. Also, blood circulates through the test to the periphery and respiratory exchange may occur by diffusion across this surface. In all the experiments described, a large surface of sea water has been exposed to the air so that gaseous diffusion alone should serve to keep the water fully aerated, but vigorous air currents have also been added, to ensure both aeration and constant circulation. However, when the latter was deliberately omitted, the frequency of squirting was not affected. The total volume available for feeding is the sum of the through-current and the volume driven by the branchial siphon, i.e. about 150 c.c./hr. in normal animals. If we accept the data of Moore *et al.* this is only barely adequate for an animal living on outside sea water. The economy would be severely strained if for any reason the available food concentration became further reduced. However, since the volume driven by squirting is large compared with the through-current, it is possible that squirting constitutes an integral part of the feeding mechanism and the amount squirted can be varied by changing the frequency of spontaneous squirting, a phenomenon which has been observed in *Phallusia* as described above.

THE EFFECTS OF FEEDING AND STARVATION

This interesting possibility, that the squirting is part of a controlled feeding mechanism, has been tested in the following way using both siphon-closing and flow-recording methods. Animals were left to starve in filtered sea water and then fed with mixed flagellate cultures. Alternatively, they were kept in circulation in the Plymoth tank sea water with different rates of flow. The results of the experiments were consistent and precise. After a period of starvation lasting some 15 hr. the frequency changes from the normal to the fast (Fig. 11). The addition of food restores the normal frequency in about 2 hr. After continued starvation for 2 days the frequency is ultra-fast, of the order of one every 2 min. The amplitude is considerably reduced, possibly as a consequence of the increased frequency which may produce some fatigue. Another phenomenon now appears: the siphons slowly move about and it is difficult to keep the levers on the drums. On adding food the siphons become still, and both frequency and amplitude are restored to normal in about 3 hr. (Fig. 12). Control experiments with graphite particles (aquadag S) in place of food particles failed to produce an effect (Fig. 14). In one experiment (illustrated in Fig. 13) the food was added continually and in large measure following a short period of starvation. The animal quickly produced its normal and then its slow speeds. Later, the same phenomenon which had been observed in some freshly fed animals, namely the decrease in amplitude of the contractions with the branchial siphon leading, was again recorded. Some contractile activity was still present but the amplitude was almost completely suppressed. During this time the animal was relatively insensitive o external stimuli. After 2 hr. the food concentration was reduced by mixing vith fresh sea water and the normal activity was quickly resumed.



ig. 11. Continuous records in the Plymouth tank circulation. The rate of flow was great at first and reduced at the point indicated by the arrow to a tiny trickle. An interval of 16 hr. separated the two parts of the record. Time in hr.



ig. 12. Records of an animal after 48 hr. starvation in filtered sea water. Food was given to the animal towards the end of the second part of the record, and the third part is a direct continuation following lever adjustment. An interval of 10 hr. separated the first and second parts. Time in hr.

The records obtained by the flow method show similar results (Fig. 15). addition, they show that the ciliary current is reduced (whether by aperture ntrol in the branchial wall or change of ciliary beat cannot be decided) ıring starvation, and is restored remarkably quickly in the presence of food particles. If the branchial siphon is denied free access to fresh sea water and forced to resample the same volume continually the rate of squirting goes up

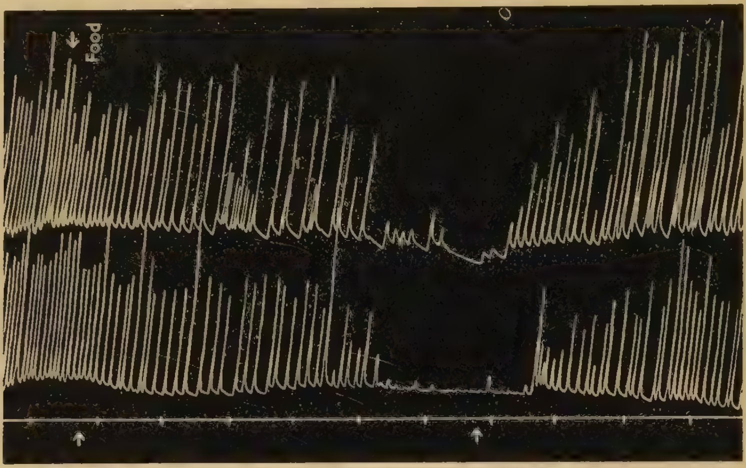


Fig. 13. Records of the activity of an animal starved for 14 hr. and given food continuously for 6 hr. from the point indicated by the first arrow. At the second arrow food was no longer given. A rich flagellate culture was used for feeding. Time in hr.

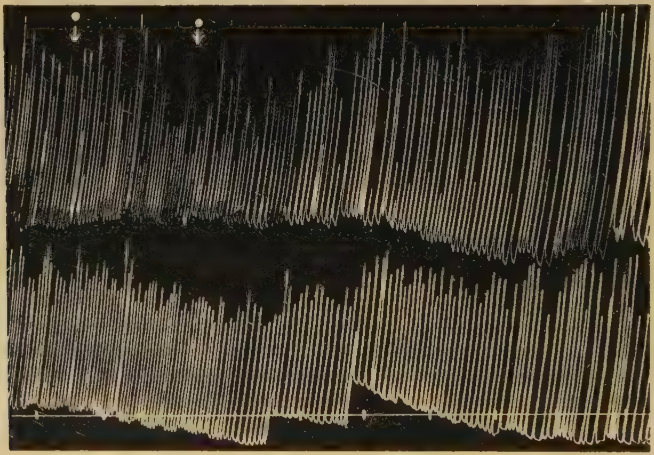
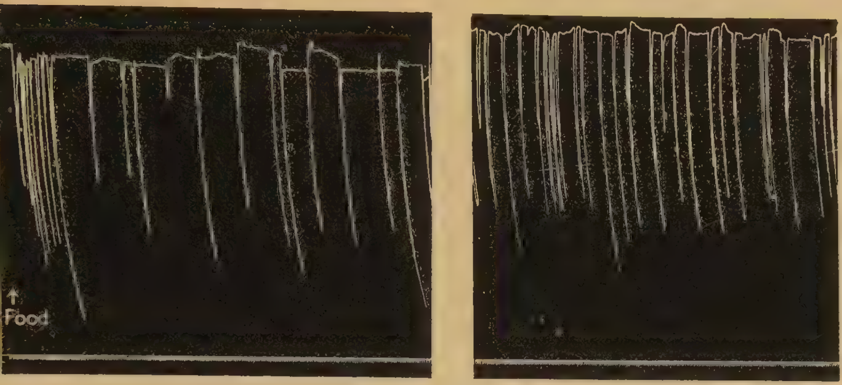
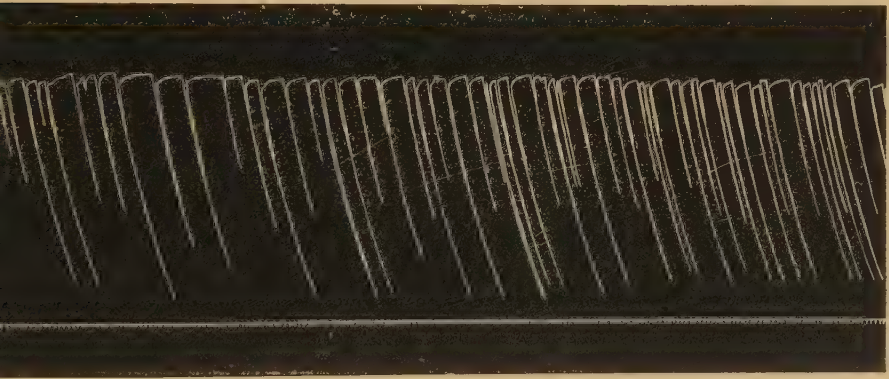


Fig. 14. Control experiments for the above (Fig. 13). The animal was fed only on graphite particles. It was given some food as well at the second arrow. Time in hr.

rapidly (Fig. 16). The ciliary current was not restricted since a suitable capillary input was used and there was no interference with the atrial squirting, hence it is unlikely that the increased rate is due to interference with re-ration, but is most probably due to the progressive depletion of food from water. It is clear from all these experiments that the frequency of spon- eous squirting in *Phallusia* depends upon the concentration of food available



. 15. Flow method recording of atrial siphon squirts immediately after feeding and 16 hr. later. The through-current is proportional to the depression below the upper edge of the record. In the left-hand figure the mean depression represents a continuous flow of 1 c.c./min.



16. Flow method recording of branchial siphon squirts showing the effect of restricting the sampling by introducing a capillary input. The arrow at the start of the record indicates a calibration depression of 10 ml. The frequency of the activity increases rapidly during the experiment. Time in min.

the branchial siphon. The frequency is low when there is a rich supply, h when the supply is impoverished. In this connexion the recent report of llier, Ray & Magnitzky (1950) is interesting. They find that the volume of effluent of oysters is proportional to the concentration of some unknown ble organic material (measured as arabinose equivalents) in the sea water.

Data on the important question of relation between feeding habits and feeding frequency and the concentration of available food over long periods are almost non-existent.

The demonstration that the rate of squirting in an ascidian depends on the concentration of food substance in the branchial sac demands an inquiry into the nature of the receptor mechanism. It has been shown that some of the particles entering the branchial sac of an ascidian enter the mouth of the ciliated pit (van Weel, 1940). Carlisle (1951) has demonstrated the same phenomenon in a salp and confirmed the observation in a monascidian, finding the particles in both the ciliated pit and the neural gland. Carlisle found a gamete-releasing mechanism in *Phallusia* in response to the presence of eggs of the same species in the food. He suggests that the reflex pathways are as follows:

Stimulus	Chemical from ingested gametes
Receptor	Neural gland
Afferent pathway	Hormonal—gonadotrophin
C.N.S.	
Efferent pathway	Neural
Effector	Gonads
Response	Release of gametes

Nervous connexions between the ciliated pit and the ganglion have been demonstrated in two forms (Metcalf, 1895; Hunter, 1898) but have been denied in others (Julin, 1881). The nature of the afferent-efferent pathways needs investigation, but there is certainly a possible receptor mechanism for the food stimulus in the ciliated pit—neural gland complex.

DISCUSSION

The work falls into two parts for the purposes of discussion: (1) the study of the physiological mechanisms of the spontaneous activity; (2) the consideration of its functional significance.

(1) The recent work of Batham & Pantin (1950) has drawn attention to the fact that long-period recording may reveal rhythmical patterns of activity in sedentary animals which on casual observation appear to be quite passive. The sea-anemone, *Metridium senile*, was shown to exhibit periodic contractions of the parietal musculature at intervals of about 10 min. The time sequence is, as Batham & Pantin say, quite low, and they compare it to the slow rhythm of the outbursts of activity of the *Arenicola* proboscis (Wells, 1949) and mammalian uterus. The normal 6–9 min. rhythm of the spontaneous squirting of *Phallusia* is also of this order and deserves equal comparison. The nature and site of the pacemaker presents a difficult problem. The nervous system is extremely simple in anemones and it is difficult to visualize how both pacing

the co-ordination of muscular movements as complex as those described Batham & Pantin can be effected by it. The *Arenicola* extrovert is paced a region of the oesophageal wall (Wells, 1937). In *Metridium* a part of the ly-wall appears to act as 'leader'. Other parts of the wall follow the ıder's' contractions. Although a leading sector may continue to set the e for a considerable time the site of the leadership may change from time time. One important aspect of the activity is now clear for both the *nicola* and *Metridium* systems: the activity is inherent. It is modified by ernal stimulation but is not initiated from without. The chain-reflex theory ctivity does not apply to either the gross activity cycles of the polychaetes he slow movements of the anemone. The patterns of spontaneous activity *Phallusia* clearly associate it with these animals in this respect. The activity ncessant under normal conditions, it is not determined by the periodic ctuations of any environmental factor or by the gross products of its activity $_2$, faeces etc. It occurs in the absence of food over long periods.

n monascidians spontaneous activity can be recorded from isolated siphons *ona* and *Styela*), and from isolated muscle strips (*Phallusia*). The inherent ivity may, therefore, be myogenic with considerable neurogenic control. is situation is perhaps comparable with that found in *Metridium* where one t of the wall leads the rest. The nervous system need only be involved in so as it effects co-ordination. The quick contractile activity in *Phallusia* olves the whole musculature synchronously, although each part probably its own activity. The co-ordination is seriously impaired in ascidians in absence of the ganglion, which also regulates muscle tone and some exes.

2) Concerning the functions of the squirting rather more can be established. e spontaneous contractions of the body-wall serve regularly to clear the austed contents of the branchial sac. They must, therefore, assist in keeping branchial mechanism clean and free from accumulated waste matter. They ally regularly clear the contents of the atrial siphon and this must assist in noving faeces and sexual products. The activity brings fresh supplies of sea er to the exposed internal body-wall and siphon surfaces and must assist respiratory exchanges. The fresh sea water brought into the branchial hon following squirts brings with it a supply of suspended food matter. *Phallusia* the volume of water moved in this way is appreciably larger than t circulated by the ciliary through-current. The frequency of squirting ies inversely with the concentration of available food. It is therefore bable that the principal function of the spontaneous activity in *Phallusia* o subserve feeding. The method works as follows. Following a spontaeous squirt a new sample of sea water, with various small organisms and d detritus in suspension is sucked into the branchial siphon. The particles swept to the walls of the sac by the internal circulation of sea water proed by the activity of cilia lining the sac and by the current being drawn

through the apertures of the sac, also produced by ciliary activity. On the walls the particles are caught in the mucus stream proceeding upwards from the endostyle and driven towards the dorsal lamina. Here the twisted cords of mucus containing the particles are driven backwards into the oesophagus. Silt and mud which has not been picked up by the mucus during the few minutes elapsing between squirts is then ejected and the cycle is repeated.

The advantages of this method of feeding are fairly clear. *Phallusia* lives intertidally in sheltered places, or commonly offshore, where the food supply is at times very rich, but subject to considerable fluctuations and often liberally mixed with mud and silt. The branchial mechanisms are particularly liable to become clogged by this estuarine dirt. Squirting helps to remove the waste which would accumulate especially rapidly in an animal with a fast through-current. It also allows a degree of control over the rate of feeding. The continuous-current method does not allow careful control of the rate of feeding when food is plentiful and perhaps being taken in at a far greater rate than can be coped with adequately. The squirting method, since its frequency can be modified, permits a range of feeding volume from zero (when the activity is inhibited after heavy feeding) to about 300 c.c./hr. during starvation. The system is economical of energy. It is now known that the quick contractions of the salps are concerned principally with feeding rather than locomotion (Fedele, 1933; Carlisle, 1950). Some, at least, of the sedentary monascidians may be regarded as feeding by a somewhat similar method of gulping or rather squirting.

The existence of spontaneous activity in several invertebrate phyla has been clearly established, and this activity is apparently always inherent. It has been demonstrated by Wells (1950) that it serves a useful purpose in the lives of certain polychaetes. It is probably equally useful in actinians, although the evidence for this is only just being brought to light, and it is hoped that the present paper will establish its usefulness to certain ascidians. This type of rhythmic oscillation of the work output of certain muscles is widespread. In addition to the groups so far discussed it has also been described in the mantle of lamellibranch molluscs (Redfield, 1917), the *Aurelia* medusa (Widmark, 1913), the sabellid retractor muscles (Wells, 1951) and the retractor pharynx muscles of *Holothuria* (Pople, 1952). Wider investigation may be expected to reveal still more instances.

The work was carried out at the Plymouth laboratory during parts of the springs of 1951 and 1952 and the autumn of 1951, whilst occupying the London University table. I wish to thank the Director and staff of the laboratory for their kindness; Dr M. Parke for supplying the flagellate cultures; and Dr G. P. Wells and Prof. P. B. Medawar, F.R.S., for reading the draft.

SUMMARY

e previous work on spontaneous activity in ascidians is briefly reviewed: re is clear evidence for the existence of spontaneous contractions of the hons of certain monascidians but no function has been ascribed to this ivity. *Phallusia mammillata* exhibits spontaneous contractions of its hons at intervals of 6–9 min. over long periods in Plymouth tank sea water. e siphon contractions are synchronous. The frequency of the contractions y shift fairly quickly under constant conditions to a higher or lower rate ich is usually twice or one half the normal rate.

\ method of recording the volume of water propelled by spontaneous ivity is described. The method also records the ciliary through-current at same time as the squirts.

The frequency of the squirting is increased during a period of partial or nplete starvation. It is reduced again by adding food cultures to the water.

The physiological mechanisms involved in the spontaneous activity are estigated and comparisons are made with the spontaneous activity exhibited *Metridium senile* and *Arenicola marina*. It is finally suggested that the ntaneous squirting of certain ascidians is an integral part of their feeding chanism.

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NERVOUS ORGANS IN THE PERICARDIAL CAVITY OF THE DECAPOD CRUSTACEA

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(Plates I–IV and Text-figs. 1–4)

he observations on nervous organs in the Stomatopoda which led to the ssumption that they might have a neurosecretory function (Alexandrowicz, 952*a*, and in press) gave an impulse to the search for similar organs in other rustaceans, the more so because during the course of the work on the nervation of the heart of these animals a peculiar arrangement of nervous ements in the pericardium had been noticed (Alexandrowicz, 1929, 1932). n examination of these previously casually observed elements has shown at they are parts of a system of fibres which are the subject of the present ccount, and for which the term 'pericardial organs' is proposed.

The observations were made on different species of decapod crustaceans ith preparations stained in methylene blue. The pericardial organs stain eadily in a solution of this dye (10–15 drops of 0·5 % solution of methylene lue in distilled water to 100 c.c. of sea water) provided they are exposed in e proper way. Some hints how this should be done are given below, as ey can be better understood when the arrangement of these elements, which not the same in various species, is known. The fixation and further treatent were the same as described previously (Alexandrowicz, 1951).

GENERAL REMARKS AND NOMENCLATURE

characteristic feature of the nerves in the pericardium is that their branches eak up into fine fibrils forming neuropile-like networks. These can be stributed either in the connective tissue ensheathing bundles of thicker erve-fibres or in plexuses spreading over membraneous parts of the perirdium. The first arrangement produces what will be called 'trunks' and e second 'plexuses' of the pericardial organs. It must be pointed out that ey are not totally separated from other nerves. In fact, as we shall see, rough the trunks may pass motor fibres and processes of muscle receptor lls. In some instances the pericardial organs are represented by neuropiles ccompanying a nerve bundle for a short distance only, but there hardly can any mistake about their belonging to the same system.

It should be added that the pericardium itself receives another set of fibres not entering into this system, viz. the fibres supplying the muscles of the pericardium. Some data about these nerves were given previously (Alexandrowicz, 1932) and will not be discussed in the present paper.

The term 'neuropile' is adopted in order to indicate a very dense and intricate arrangement of the nerve endings. It may be objected that it evokes a picture of a rather three-dimensional structure, as in the central nervous system, while in the pericardium these terminations are spread in a thin stratum (even in the trunks they are on their periphery) and, moreover, the fibrils may appear as strands running in the same direction. However, no other short designation proves to fit them better and, besides, the neuropiles in the central nervous system may have various arrangements (see Hanström, 1928, p. 44).

Observations

Brachyura

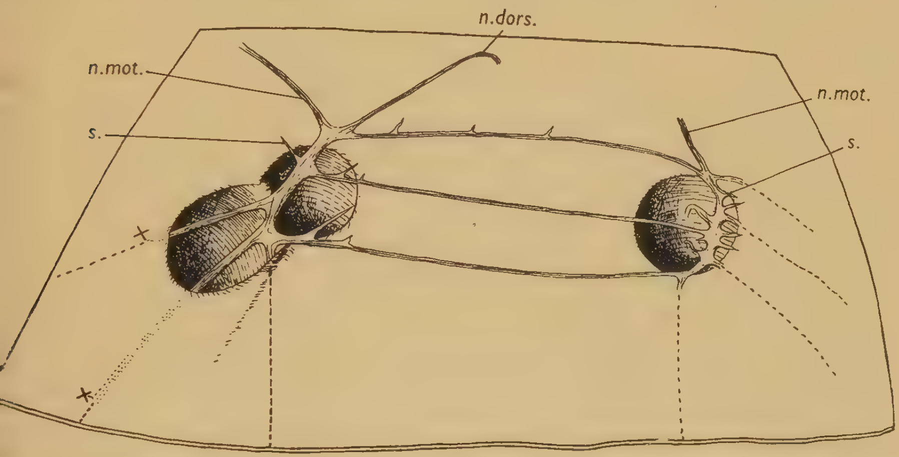
Among the Brachyura the following species were investigated: *Maia squinado*, *Cancer pagurus*, *Portunus puber*, *P. depurator* and *Carcinus maenas*. The smaller species proved to be less suitable for this purpose and after it had been found that there are only minor differences in the arrangement of the organs in all the crabs investigated the work was carried on with *Maia* and *Cancer* which were obtainable in every size required.

The pericardial organs in crabs are composed of thick nervous trunks anastomosing with each other according to a characteristic pattern. They lie on the inside of the lateral pericardium wall in such a position that their stoutest parts span the three openings of the branchio-cardiac veins. For facilitating the description these thickest parts will be called anterior and posterior bars, and those uniting the two bars will be called longitudinal trunks.

In *Maia*, the anterior bar has a comparatively simple shape, as seen in Text-fig. 1 and Pl. I, fig. 1, and the various specimens examined have shown no great deviation from this pattern. It crosses obliquely the first two openings of the veins and sends through the first of them a stout prolongation which runs far into the vein up to the point indicated by a cross in Text-fig. 1. There are two other prolongations, of smaller calibre: one running into the same vein but situated more dorsally and not extending so far, the other directed ventrally.

The posterior bar stretches opposite the 3rd opening which serves for the supply of blood from two veins which join just before they reach the pericardium. This bar has the form of a crescent and may have a more variable appearance owing to the anastomoses of its parts (Text-fig. 1; Pl. I, figs. 1, 2).

The longitudinal trunks, three in number, run more or less parallel to each other linking the two bars. Sometimes, but rather exceptionally, an additional trunk may be seen, or an anastomosing branch passes from one trunk to another. All these elements of the pericardial organs, at certain points only, are attached to the pericardial wall. These are: (i) points where the nerves connected with the trunks enter or leave the pericardial cavity; (ii) attachments of the strands which secure the position of the bars and the trunks; (iii) a part of the middle longitudinal trunk which is applied to the pericardium wall. With the exception of the latter all other parts are floating free in the pericardial cavity. The anterior prolongations are also floating in the lumen of the vein, being attached only at the points indicated.



Text-fig. 1. *Maia squinado.* Semi-diagrammatic view of the pericardial organs of the right side, with part of the lateral pericardium wall showing the three openings of the branchio-cardiac veins. The nerves running from the central nervous systems into the pericardial organs are drawn in dotted lines. The points at which the two anterior nerves pass into prolongations of the bar situated in the lumen of the vein are indicated by crosses. *n.mot.*, nerves running to the muscles; *n.dors.*, dorsal nerve of the heart; *s.*, strands suspending the trunks.

Connected with the trunks are: (i) nerves coming from the central nervous system; (ii) nerves arising from the trunks but not belonging to the system of the pericardial organs; (iii) strands securing the trunks.

The nerves from the central ganglia pass into the bars. The anterior one is joined by three nerves. The first and the second run into two prolongations situated in the anterior vein, the third runs along the vein of the second opening. How many nerves join the posterior bar is more difficult to determine. One can see five or even six of them approaching the bar from the ventral side and from behind. One stout nerve runs in the wall of the vein. It may be that some are composed exclusively of fibres which only pass through the bar to the muscles, others may be branches of the same segmental nerve. It may be assumed that no less than four segmental nerves reach the posterior bar, but this estimation is not certain.

The nerves coming from the central nervous system and entering the bars carry also fibres which have a different destination. These are: (i) fibres innervating the muscles situated at the dorsal edge of the thoracic epimera (*n.mot.*, Text-fig. 1, Pl. II, fig. 6); (ii) fibres running to the heart as a nerve called n. cardiacus dorsalis (Alexandrowicz, 1929, 1932) (*n.dors.*). The former are sometimes distinctly seen simply to pass through the bars without entering into relation with their fibres (*mot.*, Pl. II, fig. 5). The heart nerves, however, pass through that portion of the anterior bar in which the fibres are intermingled, and I have so far been unable to obtain adequate evidence whether or not the heart nerves have any connexions with those of the pericardial organs.

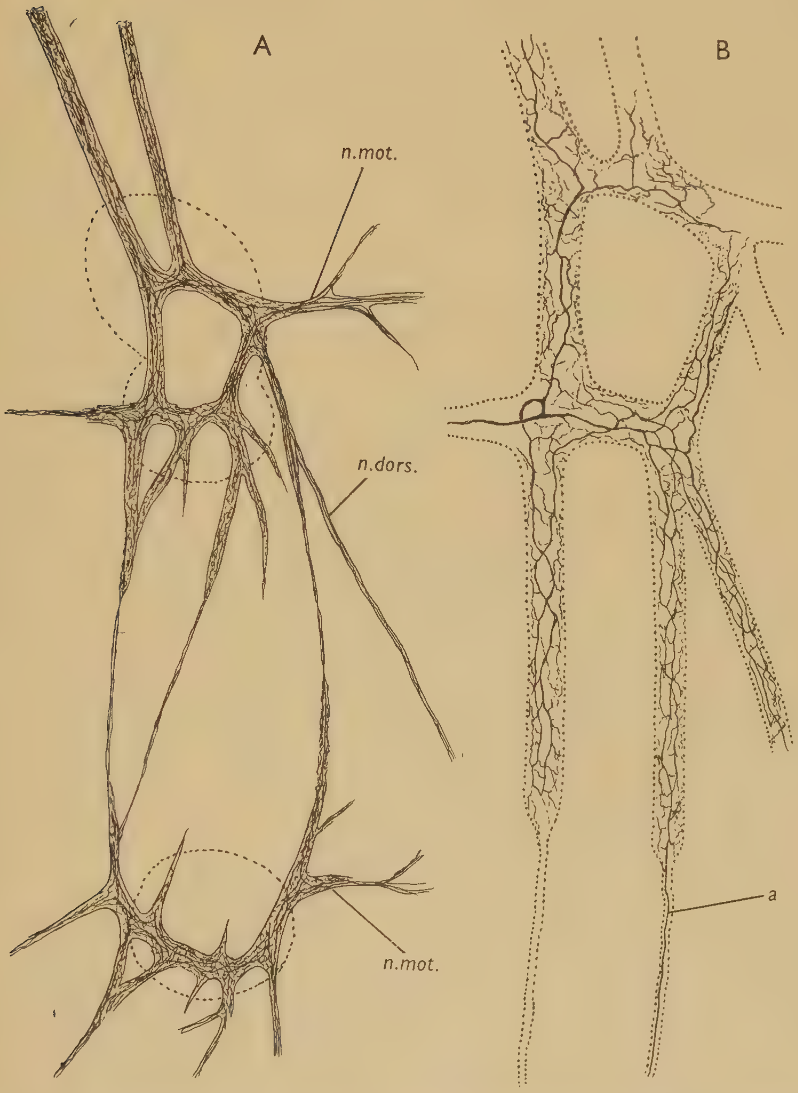
Near the third opening branches may be seen arising from the posterior bar and the middle longitudinal trunk, ramifying in the vicinity of this opening and also passing on to the wall of the vein (Pl. I, fig. 2). Some of these branches apparently spread in the pericardium wall, others pass to the muscle fibres. The branches ramifying in the pericardium do not show the characteristic terminations of the pericardial organs and one may be in doubt whether they end in the connective tissue or in the underlying muscle fibres.

The short tapering offsets of the trunks which secure the suspension of the trunks have a special character. They consist of connective tissue covered by strands of neuropiles among which a few fibres of a little stouter calibre may be noticed. They spring from various points of the bars, with the exception of the prolongation of the anterior bar into the vein, and of the longitudinal trunks, with the exception of that part of the middle one which is applied to the pericardium wall.

It may be mentioned that at the dorsal end of the anterior bar there is a nerve cell with long processes, one of which, the axon, passes through this bar towards the central ganglia (*n.c.*, Pl. II, fig. 6). This cell belongs perhaps to a special category of muscle receptor cells found in the Macrura and which will be mentioned later. In *Maia* I have noticed only one cell in this region and I was unable to ascertain where its processes end.

In other crabs the arrangement of the pericardial trunks shows some differences. In *Cancer pagurus*, instead of a single anterior bar, there are four or five short ones joined in the manner shown in Text-fig. 2A. Two thick prolongations pass through the first opening into the branchio-cardiac vein. The posterior bar is more or less similar to that of *Maia*. The longitudinal trunks are more irregularly arranged than in *Maia*, for, starting from the anterior bars, they may divide and send branches into another trunk, or two of them may unite into one (Text-fig. 2A; cf. Pl. I, fig. 4). Only the anterior and posterior portions of the longitudinal trunks are floating free, since for about half or more of their total length they are embedded in the tissue of the pericardum wall where they are deprived of the neuropile sheath.

In *Portunus puber* and *P. depurator* the arrangement of the anterior bars is



Text-fig. 2. *Cancer pagurus*. A, pericardial organ of the right side. The outlines of the openings of the veins are indicated by a dotted line. Note the different appearance of the longitudinal trunks losing their neuropile sheaths in the middle of their course (cf. Pl. I, fig. 4); *n.mot.*, motor nerves; *n.dors.*, dorsal nerve of the heart. B, ramification of one fibre entering the anterior bar; *a*, fibre running towards the posterior bar. Finer ramifications are not represented.

about the same as in *Cancer*, only the bars appear somewhat longer. The two anterior prolongations, of which the ventral one is particularly thick, are of equal length. There are three longitudinal trunks, as in *Maia*, but here the middle trunk is free and retained by suspending strands while the two others are partly embedded in the pericardial tissue. The suspending strands of the posterior bar are particularly numerous.

In *Carcinus maenas* the bars are more or less similar to those in *Portunus*. From the longitudinal trunks I have seen only two, and both were free. Because of the difficulties in obtaining undistorted preparations from these animals only a few observations were made.

Structure of the trunks. Apart from those nerve elements which pass through the trunks to other destinations, the pericardial organs are composed of fibres which branch in the trunks themselves up to their last terminations. After entering the bars each of these fibres gives off branches which pass into the prolongations of the bars and the different trunks and, running for a long distance, send subdivisions in various directions (Text-fig. 2B). A result of this distribution of branches is that each part of the pericardial organ has fibres belonging to various neurons, and it appears as if this intermingling of the elements of several neurons in the same area were of some particular importance for the functioning of the organ. Although the individual fibres show well, especially in *Cancer* (Pl. II, figs. 7 and 8), and may be traced for a longer distance, it has not been possible to ascertain the distribution of all their branches. Neither could I tell for certain how many of these fibres come from the central nervous system. As two or three of them may be found at the entrance of each of the nerves into the trunks their total number may be estimated to be about twenty, but, as usual, one cannot be sure that some of them remain unstained and invisible.

A further question to which no satisfactory answer can be given is whether the fibres in the trunks are all alike, or there are different sorts of them as in *Squilla mantis*. To this problem I shall return later.

All the fibres, after many subdivisions, break up into fine fibrils which, as stated before, are densely arranged as in a neuropile. In methylene-blue preparations they are usually deformed into small granules, and by this mass of fine dots the pericardial organs may be recognized in all decapod as well as in the stomatopod crustaceans. There is no doubt as to their nervous nature, since the transformations of the fine fibres into small beads can be often observed, but nothing can be said about the histological structure of such ostensibly deformed elements.

In the trunks which are cylindrical in shape the various fibres are so arranged that the thicker are nearer to the axis with their branches approaching the periphery at which the neuropiles form a superficial layer. This arrangement can be best observed in *Cancer*. All the fibres are held together by connective tissue which does not stain with methylene blue. In sections it may be seen that this tissue fills the spaces between the nerve fibres, forming concentric layers around the thicker of them. The nerve fibres are not so tightly packed as in the lamellae of *Squilla*. Apart from scattered nuclei, seemingly belonging to the connective tissue and the neurolemma, there may be seen cells with granular cytoplasm showing the characteristic features of amoebocytes and which are evidently brought into the trunks by small arteries. Except for these cells no elements in the trunks could be noticed having special affinity to eosin, acid fuchsin, or dyes entering in the Azan-mixture.

The pericardial organs in crabs are easily accessible. After removing the carapace, the dorsal pericardium wall, and cutting through the dorsal ligaments of the heart, one can see the pericardial organs spanning the openings of the veins or applied to the wall near to these openings, the latter position evidently due to the adherence of blood clots to the organs and the neighbouring parts. As these organs are attached at few points it is possible to cut them out, but for staining it is better to cut out the epimeral plates leaving the organs *in situ*. The preparation, after trimming the protruding parts, should be fixed to the paraffin plate and put into methylene-blue solution. The removal of the chitin and the muscles should be made later, during washing after fixation. The chitin then detaches quite easily. Final cleaning up, as removing the last of the muscle tissue and cutting out parts of pericardium membrane obstructing the view, can be made when the preparations have been taken to xylol.

The pericardial organs can also be approached from the ventral side. It is then advisable, when removing the carapace, to leave in place the membrane covering the pericardial cavity from the dorsal side and cut out the epimeral plates with the heart attached to them. After stretching the preparation with its inside uppermost the ventral pericardium wall (pericardial septum) should be cut through on each side of the heart, preferably with the adjoining half of the ventral heart wall, and stretched so that the pericardial cavity is well exposed from the inside. This method has its advantages for the observation of the nerves passing through the pericardial trunks to the muscles and to the heart. The latter nerve, however, is elusive and there are some difficulties in tracing it up to the heart.

Eupagurus bernhardus

In *Eupagurus* the most conspicuous parts of the pericardial organs appear as two trunks curving along the sides of the heart as shown in Text-fig. 3A (cf. Pl. III, fig. 9). These trunks vary in thickness and in some places divide into strands which unite again. Trunks of smaller dimensions connected with these main trunks are of two sorts: some conveying fibres from the central nervous system, others being branches given off by the trunks. It is often difficult to distinguish one sort from another, since, at the points where they join the main trunks, fibres from the trunks may pass into the nerves of central origin to run in the same nerves in the opposite direction. They can be identified if it can be stated that farther away they leave this nerve, but more than often after seeing a portion of such mixed nerve one remains in doubt in which direction its fibres may run. Moreover, as the branches of the trunks tend to anastomose in a plexus the tracing of fibres is very uncertain.

It may be assumed that no less than five segmental nerves are sent by the central ganglia to form the pericardial organs but possibly there are more of them. It is also uncertain whether they all belong to the thoracic segments since one of them coming from behind can be followed so far backwards that its origin in the 1st abdominal segment is probable.

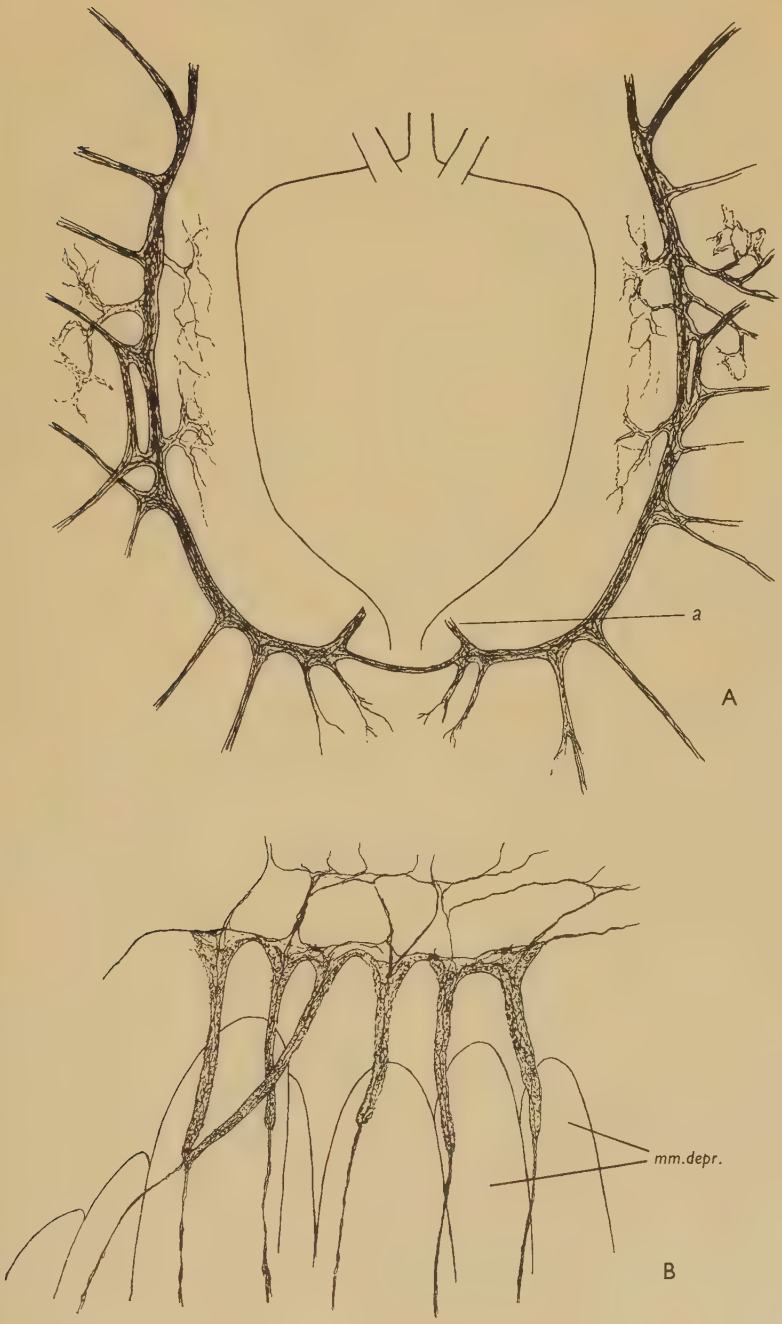
The branches arising from the trunks are of various lengths. Some at a short distance form a plexus spreading over the lateral pericardium wall which as a thin membrane covers the muscles inserting into the epimeral plate; the longer ones pass on to the farther parts of the pericardium and to the heart ligaments. An anastomosing branch links the posterior ends of the main trunks (Text-fig. 3A).

The fibres arising from the trunks and from their branches have various destinations. Some of them run to supply the muscle fibres which in the pericardium of *Eupagurus* are more abundant than in other species investigated. Others ending in neuropiles can be regarded as the elements of the pericardial organs. Their arrangement and distribution is much less uniform than in crabs, since they are present not only in the form of the trunks but as strands extending plexus-like over the pericardium wall or accompanying branches of the nerves.

Nor in *Eupagurus* was it possible to determine the areas of expansion of individual neurons, although the nerves stain well, and this animal would perhaps be the most suitable for this kind of investigation were it not so difficult to obtain preparations in which the trunks could be observed for a sufficient distance. The details which could be noticed confirm the observations made in crabs. Thus it can be seen that one fibre gives off branches passing into different parts of the trunk (Pl. III, fig. 10). It can also be stated that the nerves coming from the central nervous system each carry more than one fibre to the pericardial organs, and that the fibres brought in by different nerves overlap each other in their terminal distribution. The presence of an anastomosis through which the fibres pass into the trunks of the opposite side shows that in the neuropiles the contralateral neurons also take part.

The structure of the trunks resembles greatly that described previously in crabs, the main difference consists in that the trunks in *Eupagurus* are not cylindrical but more flattened, and that there is less connective tissue between the fibres. Otherwise the branching of the thicker fibres resulting in forming the neuropiles appears to follow the same pattern.

In *Eupagurus* it is better to expose the pericardial organs approaching them from the ventral side. After the thorax has been opened from this side and the gonads and digestive organs removed, the preparations should be spread so that the pericardial septum is well exposed. It is advisable not to try to open the pericardial cavity before the trunks of the pericardial organs, which stain readily even when covered by this membrane, become visible. Even without taking away this membrane they show fairly well in mounted preparations. In any case good preparations are difficult to obtain, for on removing the chitinous parts the pericardial organs are easily damaged or displaced.



ext-fig. 3. A, *Eupagurus bernhardus*. Pericardial organs flanking the heart. *a*, branches running in the dorsal direction, shown as cut. B, *Leander serratus*. Pericardial organs of the right side. *mm.depr.*, musculi depressores. Other muscles in this region are not represented.

Leander serratus

In *Leander* the pericardial organs are composed of five or six trunks which run in the pericardial cavity near to its lateral wall in the dorsal direction and curving arch-like become linked to one another. Their arrangement is shown in Text-fig. 3B and in the photograph (Pl. III, fig. 11). Account should, however, be taken that in the living animal the trunks are not all in the same plane and, besides, in the flattened preparation reproduced in the photograph they are somewhat deformed.

No less than five segmental nerves contribute in forming these organs. Here again the exact number of these nerves is uncertain, although in *Leander* it is easier than in other species to observe the courses of these nerves even up to the ganglionic cord. The nerves have a mixed composition for they contain fibres: (i) to the pericardial organs; (ii) to the pericardium muscles; (iii) to the ordinary muscles; and (iv) to the heart. In addition, three or four of them carry axons of muscle receptor cells situated on the inside of the epimeral plate. These cells are presumably the same elements as the so-called '*N*-cells' in *Homarus* and *Palinurus* (Alexandrowicz, 1952*b*), but in *Leander* they are comparatively much larger. They lie quite near the trunks of the pericardial organs, but do not seem to have any relation to them other than using them as their route towards the central nervous system.

Although these animals because of their size are less suitable for this kind of investigation and it is somewhat difficult to make preparations, certain features can be better brought to view in *Leander* than in other crustaceans e.g. those points where the nerves running from the central nervous system become surrounded by the neuropile sheath (Pl. III, fig. 12). The appearance of the trunks is so characteristic that, although their arrangement is different, they doubtless have to be regarded as belonging to the same category as the organs of the species described above.

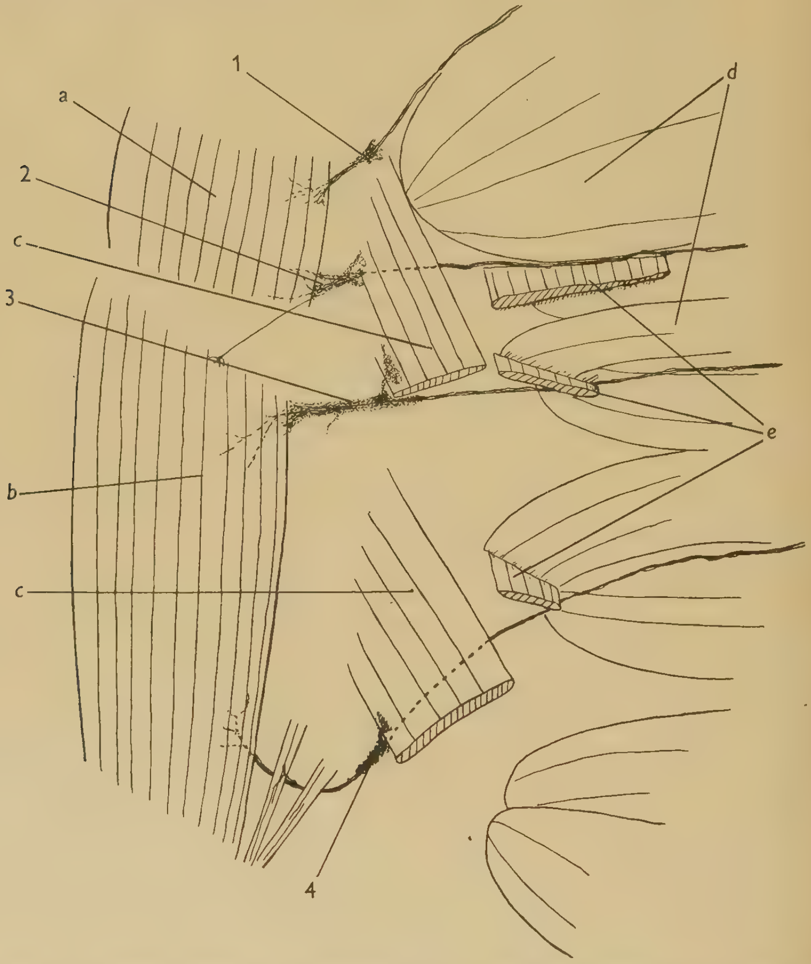
To expose the pericardial organs in *Leander* the carapace should be removed and the digestive organs and gonads taken out. All parts of the animal, excepting the thorax with the first abdominal segment left with it, should be cut away. After sectioning the thorax into two parts—for observation of the course of the nerves into the central ganglia the section can be made asymmetrically—each half has to be attached with the inside uppermost, care being taken not to injure parts of the epimeral plate near its dorsal margin. The thoracico-abdominal muscles stretching from the 1st abdominal segment to the epimeral plate should be pulled aside so as to leave the epimeral plate exposed as far as possible. In animals which had been injected with methylene blue the pericardial organs may be distinctly visible. If not, the preparations should be put into methylene-blue solution. If, after some time, when the trunks become recognizable, it is noticed that they are not well exposed, this may be corrected by stretching the preparation properly or by removing the tissues hindering the staining.

Homarus vulgaris

The pericardial organs in *Homarus* are less conspicuous than in the species viously described, for the fine neuropile-like terminations mostly spread plexuses forming a very thin layer on the pericardial wall and on heart ments, and in but a few places and for a short distance only do they form aths surrounding the nerves passing through the pericardial cavity. The ves around which such sheaths are present, and which in these parts have appearance of the trunks of pericardial organs, are the 1st to 4th thoracic ves (so termed because they come from the 1st to 4th thoracic ganglia, ugh in fact belonging to the 4th to 7th thoracic segments). On the 1st and of these nerves the neuropile sheaths are very short, especially on the mer where its presence in many instances seems to be doubtful. On the l and 3rd they are quite distinct (Pl. IV, fig. 13). The trunks can be best ntified at points where they reach the muscles lying on the inside of the meral plate (Text-fig. 4). The 1st and 2nd at the ventral edge of the contractor epimeralis, the 3rd at the anterior ventral corner of the 2nd d of the lateral thoracico-abdominal muscle, the 4th ventrally of the essory bundles of the same muscle. All trunks stretch between these nts and the edge of the dorsal thoracico-abdominal muscles which bulge the pericardial cavity. The topography of the muscles was described viously (Alexandrowicz, 1952*b*). The names of those shown in the figure l not mentioned in the paper quoted are those adopted by Schmidt (1915). The 2nd trunk may appear as composed of two or three parts (Pl. III, 13); the 3rd, the longest of all, sends strands inserting into the epimeral te obviously serving the same purpose as similar strands securing the pension of the trunks in the Brachyura (Pl. III, fig. 14).

Apart from motor fibres which are of stout calibre, there pass through the nks, as in *Leander*, the axons of the muscle receptor cells. In the descrip- n of these elements in which they were termed *N*-cells (and designated by numbers 1–5) the course taken by their axons was not specified. Now ould observe that cell no. 3 sends its axon through the 1st trunk, no. 2 ough the 2nd trunk, and no. 1 through the 3rd trunk, into the 1st to 3rd racic ganglion respectively, i.e. they belong to the 4th to 6th thoracic ments. Through the 4th trunk pass the axons of the two cells of the muscle eptor organs of the 7th thoracic segment. No relation has been noticed ween the axons of receptor cells and of the motor nerves to the elements of pericardial organs.

The structure of the trunks is very much the same as in other Decapoda. differences depend on the comparatively stouter calibre of the motor es passing through the trunks; the neuropile sheath in the 1st trunk may as if it it were formed only at the point where the nerve fibres start anding on the connective tissue membrane. The quantitative relation of the nerve elements may be expressed in such a way that in Brachyura the pericardial organs form trunks through which pass nerve fibres of other sorts, whereas in *Homarus* and *Leander* the elements of the pericardial organs are attached to the nerves passing through the pericardial cavity.



Text-fig. 4. *Homarus vulgaris.* Topography of the pericardial organs of the left side in relation to the muscles inserting into the epimeral plate. 1–4, 1st to 4th trunks of the pericardial organs. *a*, m. contractor epimeralis; *b*, m. lateralis thoraco-abdominalis (2nd head); *c*, mm. dorsales thoraco-abdominales; *d*, mm. depressores; *e*, mm. thoracales anteriores.

The small dimensions of the trunks of pericardial organs may perhaps be explained by their topographic relations. There is in *Homarus* only a narrow space between the epimeral plate and the dorsal thoracico-abdominal muscles ich pass dorsally from the pericardial septum and lie between this plate d the heart. Since the pericardial organs in the form of trunks need to at free, as seems to be essential for their function, there is no room for longer trunks, nor presumably can they extend dorso-ventrally as in *ander*.

The diminutive dimensions of the trunks in *Homarus* are compensated by ich expansion of nerve fibres arising from these trunks and spreading over ge areas of the membraneous tissue lining the lateral wall of the peri- dium and forming the heart ligaments. The photograph (Pl. IV, fig. 16) ows such fibres starting from the 3rd trunk, but only the thicker ones are en fairly well. Actually, strands of the finest filaments spread in plexuses tween these fibres and are so densely arranged that at many places little ace is left uncovered by fine granules in which the fine fibrils are disinte- ting. The nerve elements of this kind appear to be particularly abundant the vicinity of the trunks. On the dorsal and ventral wall of the pericardium y seem to be missing. This, however, does not imply that the ventral wall deprived of nerves for, as stated before, there are nerve fibres running here the muscles of the pericardial septum and to the arterial valves (Alex- drowicz, 1932).

ints about dissection and staining of the nerve elements in this region have been en in the paper quoted dealing with the muscle receptors in the thorax. The nique is much as with *Leander*, i.e. to approach the nerves from the dorsal side to turn the epimeral plate in such a way that it becomes exposed from the de.

t is also possible to observe the trunks in their natural position. After the dorsal l of the pericardium has been removed the dorsal thoracico-abdominal muscles st be pulled a little aside. Then, through the cleft between these muscles and the neral plate, the openings of the branchio-cardiac veins come into view, and tched above them the 2nd and 3rd trunk of the pericardial organs may be seen. e identification of these trunks may, however, be uncertain, as strands of connective ue and blood clots may stretch in a similar way. The 1st and the 4th trunk may be found, but here the mistakes are more likely to occur. In stained preparations re is no difficulty in distinguishing the nerves from other elements.

Isopoda

n the pericardial cavity of *Ligia oceanica* I noticed two nerve trunks nning in a longitudinal direction alongside the heart tube which do not w connexions with other elements innervating the heart and the muscles the pericardium. It is therefore probable that they might be pericardial ans similar to those in decapods and stomatopods. However, no satis- tory evidence could be obtained, chiefly because of difficulties in exposing parts of the pericardium for staining. Moreover, these trunks do not n well, and in the animals of such a small size the character of nerve fibres difficult to ascertain.

Palinurus vulgaris, Scyllarus arctus, Astacus fluviatilis

These species have not been examined in the course of the present work, but a mention of observations previously made may be not out of place. In all of them I noticed, when investigating the innervation of the heart, the existence of 'peculiar terminations of the nerves entering the pericardial cavity'. These were doubtless the elements of the pericardial organs, as follows from the statement: 'the thin fibrils which originate from the thicker fibres present a mass of blue points like a "punctate substance" of the neuropiles.... Similar structures can be seen on the epimeral plate and I had previously observed them in *Potamobius*' (Alexandrowicz, 1932, p. 230). In *Astacus fluviatilis* (then temporarily called *Potamobius astacus*) I had, moreover, described and pictured a peculiar arrangement of fibres around the dorsal nerves of the heart ending in irregular plates or cell-like bodies (Alexandrowicz, 1929). I called it 'apparatus nervi dorsalis', suggesting that it possibly might be concerned with the appreciation of the pressure in the pericardial cavity. In the light of the later findings it seems more probable that it belongs to the system of the pericardial organs, and as a similar body has not been found in other Decapoda it might be of interest to examine it from this angle.*

DISCUSSION

It has been shown above that the two forms of arrangement of neuropile-like endings, viz. as sheaths round the bundles of fibres or spread in plexuses over a larger area, may predominate in one group of the Crustacea and play a minor role in another. The one extreme is represented in the Brachyura, which possess remarkably large trunks and not any, or at most few, neuropile plexuses. On the other hand, in *Homarus* the plexuses are widely spread and the trunks are greatly reduced. A question arises: has this different arrangement some bearing on the function of these organs, or is it rather accidental? The morphological evidence is not unequivocal. It may be assumed, as already suggested, that purely topographical conditions are decisive in developing one or the other form. In the crabs, in which there is a wide space in the pericardial cavity, the trunks are free to attain large dimensions; while in *Homarus*, because of the narrowness of space, the conditions for functioning of the longer trunks are unfavourable and consequently the fibres have to spread out. However, this simple and plausible explanation seems not to tally well with the fact that in the pericardial cavity of *Squilla* both sorts of neuropiles are present, and apparently there is no such lack of space pre-

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* Since sending this paper to the printers I have had the opportunity at Naples of observing the pericardial organs in *Eriphia spinifrons*, *Dromia vulgaris*, *Pagurus striatus*, *Lysmata seticaudata* and *Penaeus camerote*. In each of these species the pericardial organs are arranged according to a particular pattern differing to varying degrees from those described above. In *Penaeus* the plexuses spread over the pericardium wall attain the largest dimensions of all species investigated.

nting the development of the first sort. (In *Squilla* these have been signated 'lamellae', but they are nothing else than short flattened trunks.) ius, since the two forms of neuropiles exist in the same segments and not : from one another, it might be supposed that their functions may not be entical.

Another question which arises on comparing the pericardial organs in capods with those in stomatopods is the origin of their nerve fibres. In *uilla* they have been found to come from two different sources: one set is ven off by nerve cells lying in the abdominal segments outside the ganglionic rd, while the second set, of obviously different kind, originates in the fore-rts of the central nervous system. Peripheral nerve cells taking part in the rming of the pericardial organs have not been found in decapods, and it is be assumed that the corresponding elements became centralized. Unless, wever, the structure of the pericardial organs has not at the same time come simplified in the Decapoda, the second set of fibres should be present them. However, the histological examination does not afford evidence to id sufficient weight to this assumption. There may in places be seen fibres somewhat different appearance and, in particular, I tried to trace those in : anterior prolongation of the bar (in the Brachyura). But the fibres mix in ch a way that no certainty about their special behaviour could be obtained. iis, of course, does not imply that similarly looking nerves should be all of : same kind, and that the pericardial organs in the Decapoda carry no res of different origin which might have different physiological functions.

In approaching the problem of the function of these organs, it ought to be iphasized that exposure to the blood stream coming from the gills must be paramount condition for their activity. The trunks are not only in these very ices when the blood passes into the pericardium, but they are suspended in ch a way that they can be bathed on all sides by the blood. How essential must be for the neuropiles to be in the closest touch with the blood can be iged by the fact that in the same trunks, as in the longitudinal trunks of ibs, they do not develop at those parts where the trunks are embedded into : tissue of the pericardium, and are abundant where they are free. In other ecies this position of the trunks is not so easily demonstrable, but whenever s possible to verify their relation to the pericardium they are found floating its cavity.

As regards the neuropiles spreading over the membranes in a thin layer, ir particular abundance at those places where the blood stream entering pericardial cavity passes, gives support to the assumption about the vital portance of this kind of topographic relation.

This conclusion affords a clue towards the solution of a problem of the iation of the pericardial organs in the Stomatopoda. In these animals the irt and the pericardial cavity extend over some fifteen segments, but the ans in question are present in five abdominal segments only, and no reason for it could previously be found. Now in stomatopods the main respiratory organs, the abdominal gills, which are five in number, are attached to the same five abdominal segments and thus the pericardial organs prove to be situated exactly where the blood from the gills passes to the heart. It is not impossible, also, that an organ of a similar histological structure which in these animals is suspended in the ventral blood sinus, described under the name of 'the transverse bar of the 6th segment' (Alexandrowicz, 1952*a*), owes its situation to the existence of the so-called thoracic gills. This matter needs further investigation, for the knowledge of the blood circulation in stomatopods is deficient and no precise data seem to have been published about the way taken by the blood passing through the thoracic gills.

In the papers dealing with the innervation of the heart of crustaceans, in which some observations relating to the nerves in the pericardial cavity were made, I expressed the supposition that they might have some sensory function. However, in view of the findings in *Squilla mantis* this supposition had to be discarded, and it was suggested that these organs release some substances into the blood. The observations made with the Decapoda provide supporting evidence for this hypothesis and, moreover, this could be confirmed by the results of experiments performed in collaboration with Mr D. B. Carlisle. They have shown that the extracts of pericardial organs, if added to the saline solution perfusing an isolated heart of *Cancer* or *Maia*, exhibit a pronounced action on the heart rhythm. It appears probable that one of the substances produced in these organs may be adrenaline or some adrenaline-like compound, as may be inferred from the fact that the extract of the organs of *Cancer* gives a distinct positive fluorescence test as well as the blood of this animal taken from the pericardial cavity (but not that from the leg arteries). However, the effect of the extract on the heart is not identical with that of adrenaline solution. Consequently, it has to be assumed that the liberated substance is either not adrenaline or the latter is mixed with some other product. Neither of the compounds so far tested, such as noradrenaline, tyramine, acetylcholine and histamine, proved to induce effects identical with those of the pericardial organs extract. The results of these experiments will be published separately.

With regard to the question which elements of the pericardial organs are responsible for their secretory activity the balance of evidence favours the view that these are the terminations of the nerve-fibres. The alternative supposition would be to ascribe this faculty to the connective tissue and consider the nerves as destined only to convey stimuli to this tissue. However, not only does it seem unlikely that connective tissue could exhibit this faculty, but the quantity of nerve elements concentrated in these organs and their predominance over other elements makes such an assumption attributing them a rather secondary role quite untenable. Therefore, all available information considered, there is justification for drawing the conclusion that the

Crustacea possess in their pericardial cavity organs which by the way of eurosecretion release substances regulating the heart-beat.

I wish to record my gratitude to Mr G. M. Spooner for his kind help in reparing the manuscript.

SUMMARY

n the pericardial cavity of the decapod Crustacea the nerve fibres end in haracteristic neuropile-like networks which surround bundles of thicker ibres or spread over the pericardium wall and the heart ligaments. It is roposed to designate the whole system of these elements as ‘pericardial rgans’.

The arrangement of the elements constituting the pericardial organs varies n different groups of the Decapoda. In the Brachyura the bundles of fibres urrounded by neuropiles form conspicuous trunks linked with each other; he main parts of these trunks are situated at the openings of the branchio-ardiac veins into the pericardial cavity. In *Eupagurus bernhardus* two main runks flank the heart giving off branches which spread in various directions nd form at some places plexuses on the pericardium wall. In *Leander serratus* he pericardial organs are represented by several trunks running near the ateral wall of the pericardium in the dorsal direction and uniting by arch-haped branches. In *Homarus vulgaris* there are four comparatively much horter trunks situated at the lateral wall of the pericardial cavity, but plexuses f neuropile-like nerve fibres spread over large areas.

The trunks of the pericardial organs are suspended in the pericardial cavity n such a position that they may be bathed on all sides by the blood passing rom the gills. The plexuses extending over the membranes are situated in laces also exposed to the blood stream. There is evidence indicating that he pericardial organs produce substances influencing the heart-rhythm. It is ssumed that these substances are liberated into the blood by a process of eurosecretion occurring at the terminations of the nerves.

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EXPLANATION OF PLATES I–IV

All photomicrographs were made from preparations stained with methylene blue, fixed in ammonium molybdate and mounted in xylol-dammar.

Plate I

Fig. 1. *Maia squinado.* Pericardial organs of the left side (cf. Text-fig. 1). Parts of the pericardium are still attached to the trunks.

Fig. 2. *M. squinado.* Posterior part of the pericardial organs. The ramifying branches end on the muscle fibres; the destination of some of them is uncertain.

Fig. 3. *M. squinado.* Part of the posterior bar showing the intermingling of nerve fibres. The small dots are elements of neuropiles.

Fig. 4. *Cancer pagurus.* Anterior portion of the pericardial organs of the right side. The trunks span the second opening of the branchio-cardiac veins. On the left upper corner is part of the first opening into which pass the anterior prolongations of the bars. Note the absence of neuropile sheaths in the lower parts of the trunks. These parts are embedded in the tissue of the pericardium. The situation of the bars is a little distorted artificially (cf. Text-fig. 2A).

Plate II

Fig. 5. *Maia squinado.* Part of the posterior bar. *mot.*, fibres of the motor nerves passing through the pericardial organs without branching in them.

Fig. 6. *M. squinado.* Dorsal part of the anterior bar of the right side. *n.mot.*, motor nerve; *n.dors.*, fibres of the dorsal heart nerve; *n.c.*, nerve cell.

Fig. 7. *Cancer pagurus.* Nerve fibre entering the anterior bar and ramifying in the trunks (cf. Text-fig. 2B).

Fig. 8. *C. pagurus.* Part of a trunk of the pericardial organs with fibres branching in it. Nearly all nerve elements seen in this and in the preceding figure belong to one neuron. They show well owing to the incomplete staining of the superficial layer of neuropiles (seen as small dots).

Plate III

Fig. 9. *Eupagurus bernhardus.* Part of the main trunk of the pericardial organs near its posterior end (cf. Text-fig. 3A).

Fig. 10. *E. bernhardus.* Part of the main trunk with a nerve fibre sending ramifications into different branches of the trunk.

Fig. 11. *Leander serratus.* Pericardial organs of the right side. The position of the trunks is somewhat distorted by spreading and mounting the preparation (cf. Text-fig. 3B).

Fig. 12. *L. serratus.* Part of the pericardial organs of the left side, showing the changing of the appearance of the nerves coming from the central nervous system at the point where they pass into the trunks.

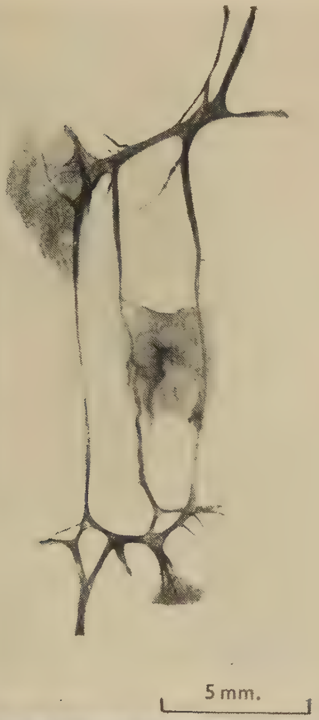
Plate IV

Fig. 13. *Homarus vulgaris.* Second and 3rd trunks of the pericardial organs (cf. Text-fig. 4).

Fig. 14. *H. vulgaris.* Part of the 3rd trunk of the pericardial organ with the strands securing its suspension (on the right).

Fig. 15. *H. vulgaris.* Parts of the trunk of the pericardial organs showing the characteristic beaded appearance of finer fibres.

Fig. 16. *H. vulgaris.* Fibres arising from the trunk of the pericardial organs (lower left corner) and spreading in one plane. The dots seen in some places are elements of neuropiles stained indistinctly.



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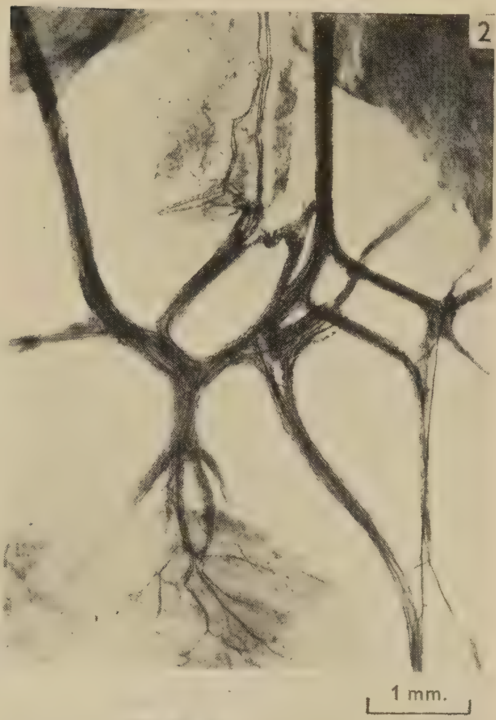

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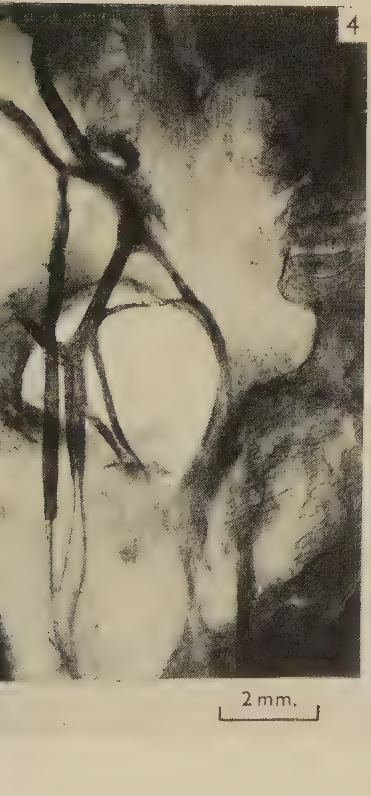

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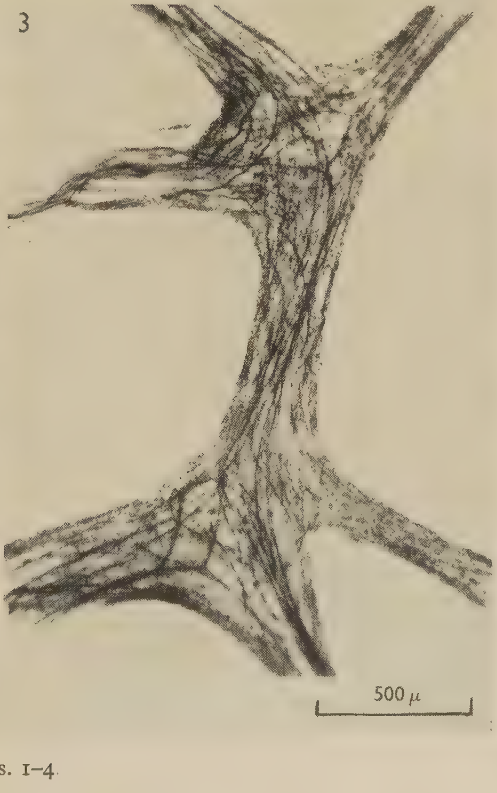

Figs. 1–4.

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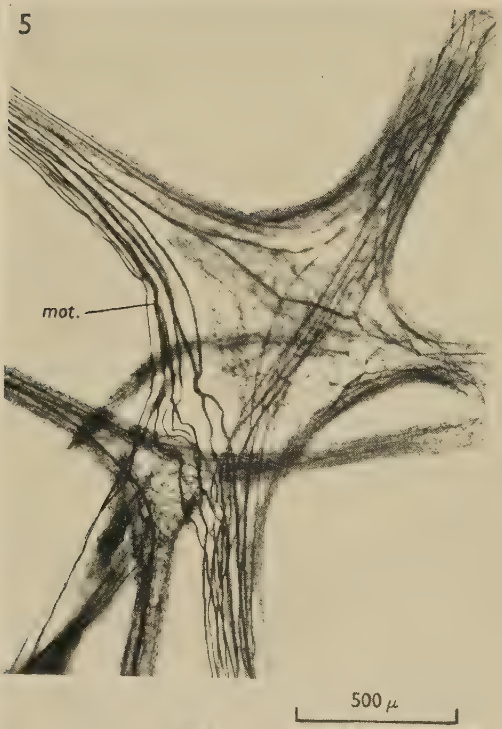

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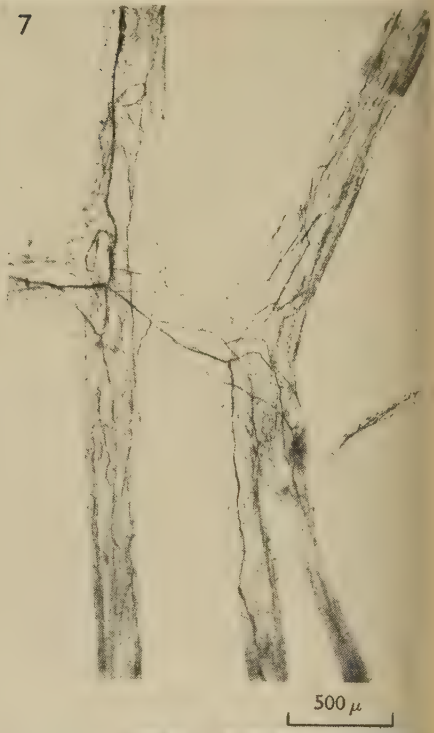

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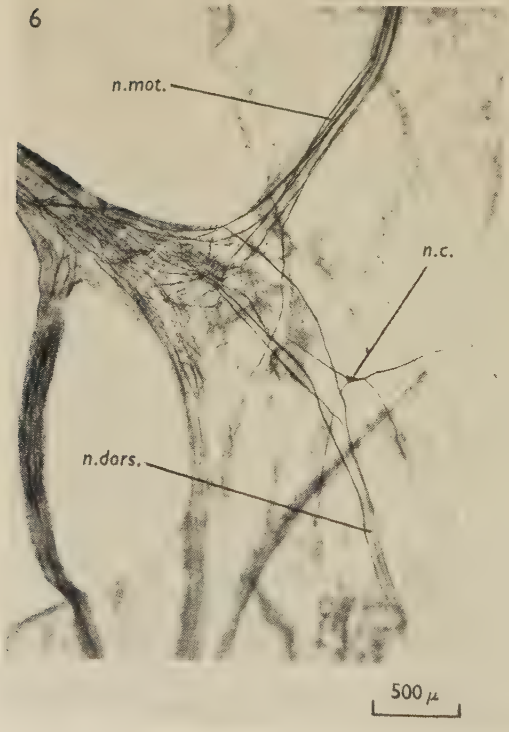
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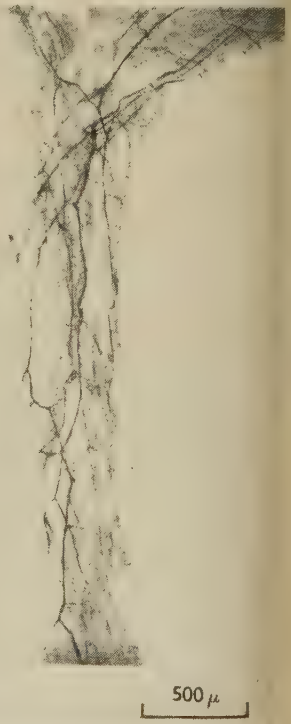
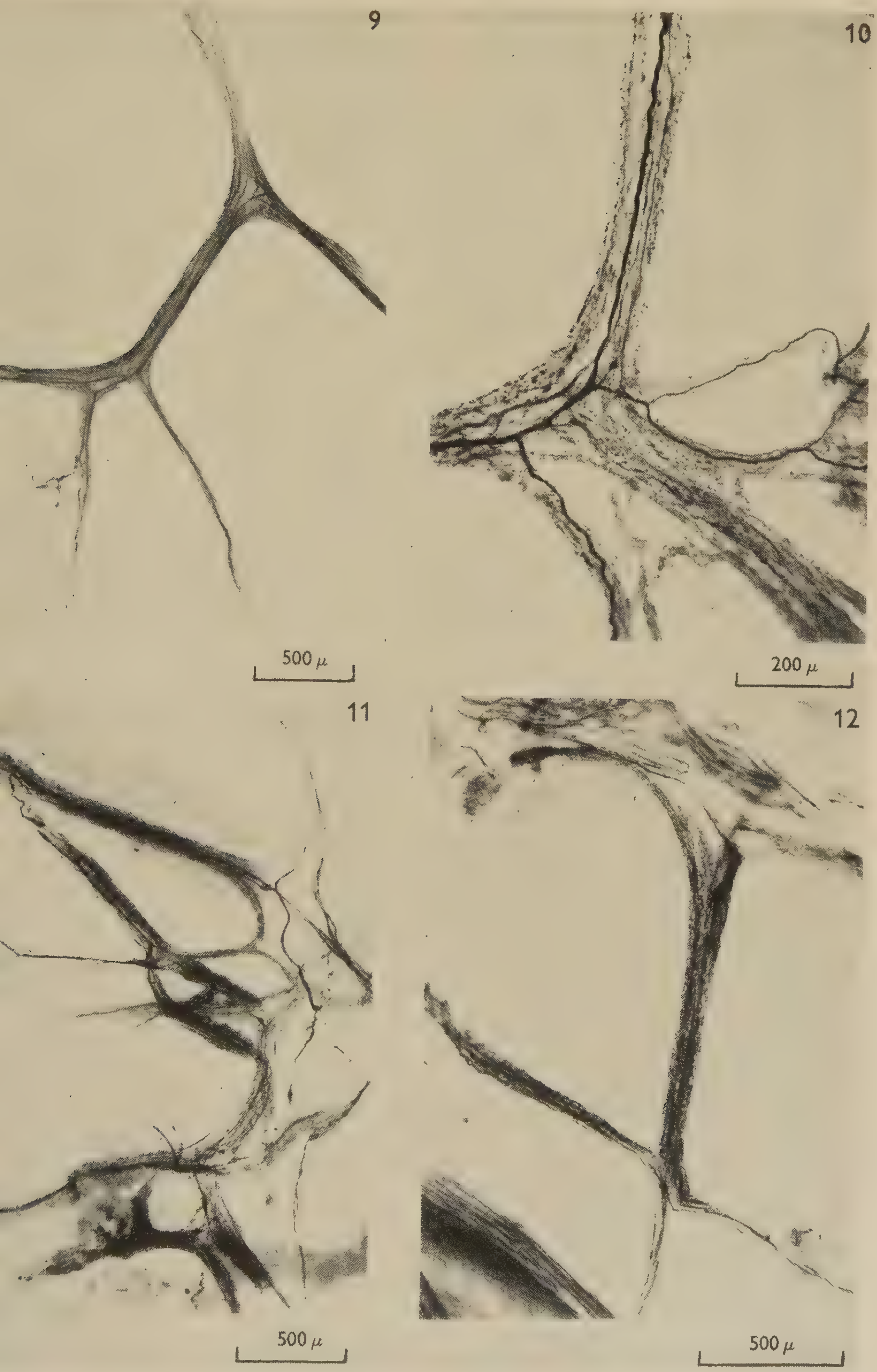


Figs. 5–8.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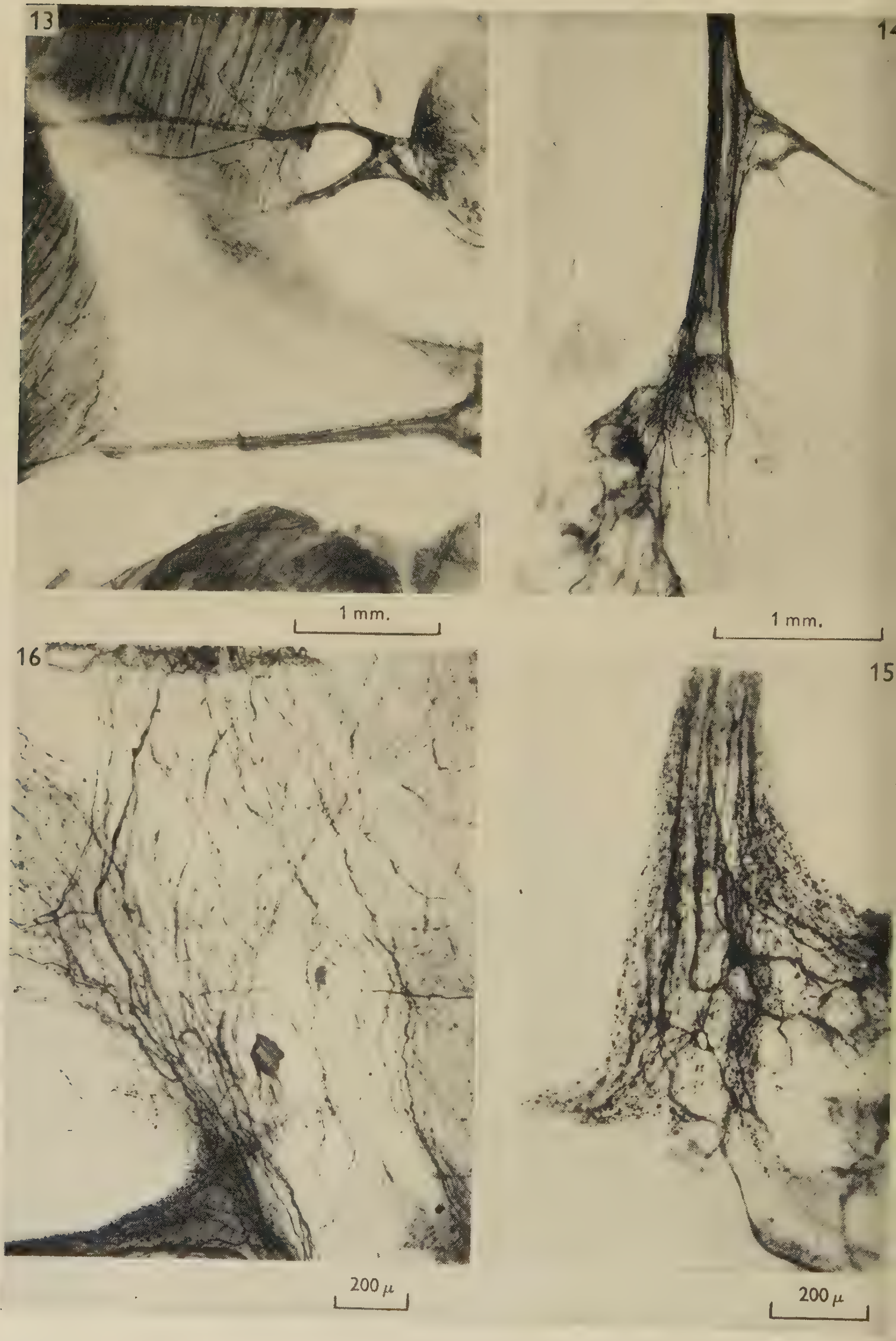
Figs. 9–12.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Figs. 13–16.

CERCARIA REESI N.SP., A NEW FURCOCERCOUS LARVA FROM PLYMOUTH

By Robert F. Hutton

From the Plymouth Laboratory

(Text-fig. 1)

A new longifurcous, pharyngeate, distome cercaria has been found to develop in the marine bivalves *Hiatella arctica* (L.) and *H. striata* Fleuriau. This larva was present in 12 of 200 specimens of *H. arctica* collected from Drake's Island during the summer months (May–August) of 1952, while the same parasite was found in 15 of 100 specimens of the limestone borer, *H. striata*, dredged from Plymouth Sound during June and July of the same year.

I first learned of this larva from Dr W. J. Rees, British Museum (Natural History), who observed it several years ago in *H. arctica* (L.). However, because of other duties both then and now, he has been unable to complete a description of this species. I am indebted to him for giving me information as to its habitat and for suggesting that I give a description of this parasite; for these reasons the present species is named in his honour. Furthermore, I am grateful to Dr W. J. Rees and Dr F. G. Rees for reading and criticizing the manuscript.

Specific nomenclature for molluscs mentioned in this paper is in accordance with Winckworth (1932 and 1951).

This work was carried out at the Plymouth Laboratory of the Marine Biological Association while the author was holding a Fulbright Scholarship.

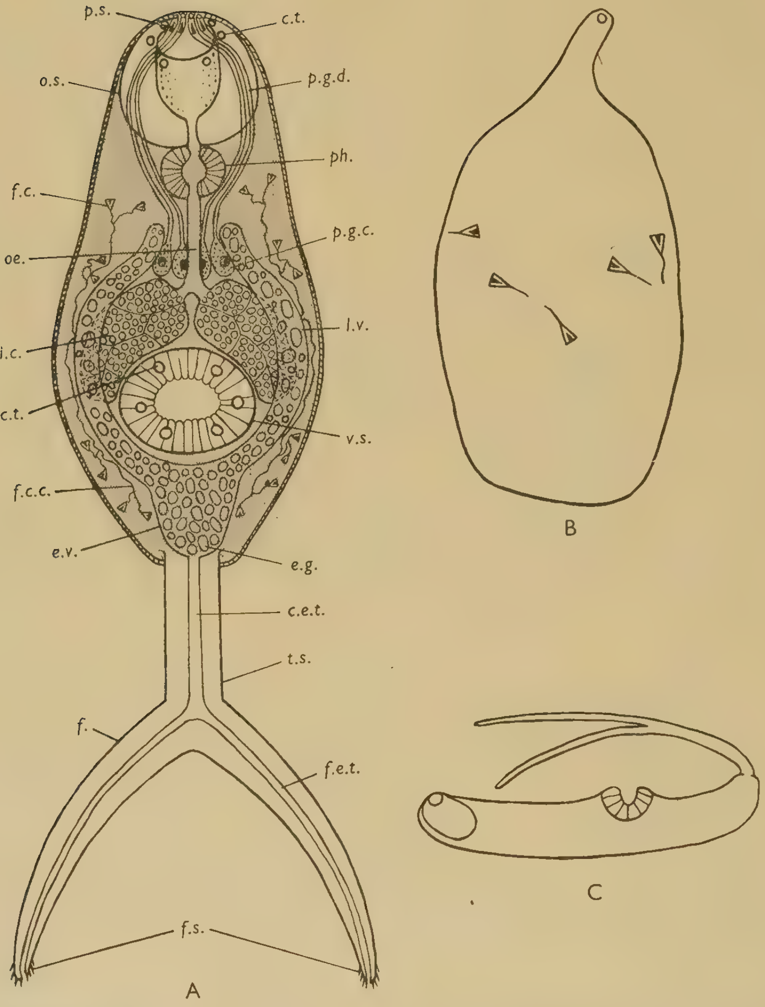
METHODS

Fully developed cercariae were obtained by isolating specimens of *H. arctica* and *H. striata* in glass tubes and allowing the cercariae to emerge. Sporocysts and developing cercariae were obtained by crushing specimens of these bivalves. Cercariae and sporocysts were studied alive and unstained, also fixed in sublimate and stained with Giemsa and with gold chloride. Measurements of cercariae were taken from naturally released living specimens slightly compressed between slide and cover glass.

OBSERVATIONS

Cercaria reesi n.sp. (Fig. 1)

Specific diagnosis. Marine, longifurcous, pharyngeate, distome cercaria developing within ovoid to sausage-shaped sporocysts. Nonoculate; body elongate, widest slightly posterior to centre of body; cuticle covering the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A
B
C

Fig. 1. A, *Cercaria reesi* n.sp. B, sporocyst. C, swimming position of cercaria. (All figures are semi-diagrammatic.) *c.e.t.* caudal excretory tube; *c.t.* cuticular tubercle; *e.g.* excretory granule; *e.v.* excretory vesicle; *f.* furca; *f.c.* flame cell; *f.c.c.* flame cell canal; *f.e.t.* furcal excretory tube; *f.s.* furcal setae; *i.c.* intestinal caecum; *l.v.* lateral branch of excretory vesicle; *oe.* oesophagus; *o.s.* oral sucker; *p.g.c.* penetration gland cell; *p.g.d.* penetration gland duct; *ph.* pharynx; *p.s.* penetrating spine; *t.s.* tail stem; *v.s.* ventral sucker.

body showing small dots arranged densely and regularly; this pattern seen in sections appears to be the surface view of fine striae passing obliquely through the thickness of the cuticle. Two pairs of penetration glands located dorsal and lateral to the posterior end of oesophagus, their ducts running forward to open to the exterior near the tip of the oral sucker; two pairs of protrusible penetrating spines located slightly behind the penetration-duct openings; oral sucker subterminal and ovoidal in shape with six semi-transparent tubercles present on its exterior surface; prepharynx absent; oesophagus of moderate length; intestinal caeca prominent, terminating lateral to the centre of the ventral sucker; ventral sucker elliptical, broader than long, with six semi-transparent tubercles present on its exterior surface. Excretory vesicle conspicuous, the lateral branches extending forward converging anterior to the intestinal caeca and terminating a short distance from the oesophagus; caudal excretory tube passes through the furcae to open to the exterior near their tips; the excretory formula is $2[(2+2)+(2+2)]=16$; tail stem attached to the body ventrally; setae on distal ends of furcae. Measurements in microns of fifty fully developed cercariae under slight pressure of cover-slip: body length 102–216 (av. 159); body width 54–100 (77); oral sucker width 30–42 (36) pharynx width 14–22 (18); ventral sucker width 30–42 (36); tail stem length 18–50 (34); tail stem width 12–26 (19); furca length 30–68 (49); and distance of ventral sucker from anterior end of body 56–92 (74). The maximum diameter of the excretory granules is $6 \cdot 8 \times 3 \cdot 0\,\mu$. Measurements in microns of fifteen living sporocysts under slight pressure of cover-slip: body length 320–800; body width 196–352.

Hosts. Hiatella arctica (L.) and *H. striata* Fleuriau.

Habitat. Sporocysts in digestive gland and gonad.

Locality. Drake's Island and Plymouth Sound, Plymouth, England.

Incidence of infection. 6 % in *H. arctica* and 15 % in *H. striata.*

Type material. To be deposited in British Museum.

This cercaria swims with its head in a forward direction and with its tail curved as shown in Fig. 1 C; this is also its normal resting position. The small dots on the cuticle of this cercaria are similar to those of *Cercaria fulbrighti* Hutton; in the latter larva spines develop from these areas. No significant difference was observed between measurements of cercariae from *Hiatella gallicana* and those from *H. arctica.*

DISCUSSION

Miller (1926) includes eight marine species in his check-list of what he considers to be all the furcocercous cercariae, excluding gasterostome cercariae, known at the time. Young (1936), reporting a fork-tailed cercaria from the Bering Sea, states: 'Thus far there have been described to my knowledge twelve marine furcocercous cercariae, at least three of which are of doubtful identity.'

Our knowledge of marine furcocercous cercariae is still very limited. In so far as the present author is aware there are but thirty-five marine forms reported in the literature. Many of these accounts are so meagre that they are of little taxonomic value. The same species has probably been designated under more than one name.

The furcocercous cercariae, other than gasterostomes, reported from British marine waters are as follows:

Parasite	Author	Reported by
Cercaria dichotoma	Müller (according to La Valette St George, 1855)	Lebour, 1908, etc.
Cercaria sp.	Jones & Rothschild, 1932	Jones & Rothschild, 1932
Haplocladus sp.	Rees, 1947	Rees, 1947
Cercaria fulbrighti	Hutton, 1952	Hutton, 1952

In addition to the above list, Rothschild (1936) notes an abnormal cercaria which possesses a 'perfectly symmetrical forked tail in a species normally possessing a simple tail'.

Cercaria dichotoma Müller (according to La Valette St George, 1855) is probably the larva of *Tergestia laticollis* (Rudolphi): most certainly it is not the same species dealt with by Pelseneer (1906) and Lebour (1908), to which both workers attributed the name *Cercaria dichotoma* Müller. Dawes (1946, p. 245) gives the name *C. fissicauda* Villot to the latter cercaria, but this does not seem likely since there are major differences between Villot's cercaria and that of Pelseneer and of Lebour. Villot reports two small teeth on the anterior border of his cercaria, yet he states that he was unable to observe a 'bulbe oesophagien'. Both Pelseneer and Lebour record the presence of a pharynx in their reports, but neither mentions the presence of any anterior teeth-like structures.

Sinitsin (1911) described a marine fork-tailed cercaria from *Abra* (= *Syndosmya*) *alba* under the name *Cercaria discursata*. While this paper was in the proof stage, a publication was received in which Uzmann (1952) described a similar larva, *C. myae*, from *Mya arenaria*. Of the marine furcocercous larvae which have been described, *Cercaria discursata* and *C. myae* appear to be most like *C. reesi*. All three species possess two pairs of penetration glands anterior to the ventral sucker. Possession of these anterior penetration glands places these forms in the 'Strigea' group of Wesenberg-Lund (1934). However, *C. reesi* appears to differ from the other two larvae by possessing two pairs of anterior penetrating spines and by possessing cuticular tubercles on the oral and ventral suckers. Although I was unable to find any mention of cuticular tubercles, or of similar protuberances, in Sinitsin's description of *C. discursata*, his figure (plate III, fig. 45) shows the ventral sucker's aperture to have a fringed outline. It is possible that this shape of the ventral sucker was produced by similar structures which various authors, including myself, have termed 'cuticular tubercles'.

At the present state of our knowledge I feel justified in separating the *Hiatella* parasite from *Cercaria myae* Uzmann and *C. discursata* Sinitsin under the name *C. reesi.*

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

THE FORCE EXERTED BY ACTIVE STRIATED MUSCLE DURING AND AFTER CHANGE OF LENGTH

By **B. C. Abbott** and **X. M. Aubert**

Journ. Physiol., Vol. 117, 1952, pp. 77–86

A study of the tension changes during enforced stretch of activated dogfish muscle has been made. Isolated coracohyoid muscles at 0° C. were stretched at constant speed during tetanic stimulation. Tension changes and work done were recorded. The events during stretch proved not to be merely the converse of those during shortening, for the tension rise during an imposed stretch depends on the distance moved and hardly at all on the speed of movement, in contrast to the changes observed during shortening. This was different from the findings of Levin & Wyman (1925) on the same muscle, and the discrepancies are discussed; but the results were similar to those obtained with frog muscle.

The readjustment of tension after constant speed stretches and shortenings was followed when stimulation was continued after movement had ceased. The tension moved toward but remained appreciably different from the isometric value at the final length; above after stretch, below after shortening; the differences increased as the speeds of movement were smaller. If stimulation was interrupted long enough for tension to drop to zero the normal isometric value was exerted when excitation was resumed. The evidence indicates that shortening or lengthening offset the internal structure of the muscle perhaps by altering some crystalline orientation and so affects the final tension at any length, giving this hysteresis-like effect. B.C.A.

MUSCLE RECEPTOR ORGANS IN THE ABDOMEN OF *HOMARUS VULGARIS* AND *PALINURUS VULGARIS*

By **J. S. Alexandrowicz**

Quart. Journ. Micr. Sci., Vol. 92, 1951, pp. 163–99

Nerve cells, connected with special muscle units which are considered to be muscle receptor organs, are described in *Homarus vulgaris* and *Palinurus vulgaris*. In each of the six abdominal segments there are two muscle receptor organs on each side, situated at the level of the superficial dorsal muscles. The muscle components of these organs are independent of the ordinary muscles. Each of them has its own nerve cell sending several processes to the muscle, and its axon into the ganglionic cord. Differences in the structure of the muscles and in the appearance of nerve cells are described, indicating that the two muscle receptor organs on the same side are of a different kind. Apart from the nerve cell each organ is supplied by at least three kinds of nerves.

In the ganglionic cord of the embryonic lobster a tract is described composed of the axons of the receptor cells. J.S.A.

Notes on the Nervous System in the Stomatopoda.
I. The System of Median Connectives

By **J. S. Alexandrowicz**

Pubbl. Staz. Zool. Napoli, Vol. xxiii, 1952, pp. 201–14

Unpaired nerve trunks running between the longitudinal connectives of the ganglionic chain have been found in *Squilla mantis*. The nerves arising from these trunks carry two kinds of fibres. The supplying of the alary muscles of the pericardium by one kind reveals their motor character. The fibres of the second kind give off numerous branches ending in neuropile-like networks which spread over the wall of the blood sinus and in the sheaths surrounding the nerves. In the sixth thoracic segment the nerves with the ensheathing connective tissue and the neuropile-like terminations form an organ in the shape of a bar suspended across the blood sinus between the flexor muscles. It is suggested that the second kind of nerves may have a neurosecretory function. J.S.A.

Vitamin C Reserves of British Troops in England and Scotland during the Winter and Spring, 1941–42

By **W. R. G. Atkins**

(Late Captain, Royal Army Medical Corps)

British Journ. of Nutrition, Vol. 5 (nos. 3 and 4), 1951, pp. 275–86

The object of the work was to study the vitamin C reserve in relation to the prevalence of gingivitis and scurvy or antecedent conditions. The general plan of investigation was drawn up by Major-General D. T. Richardson, C.B., M.C., K.H.S., Director of Hygiene. Two hundred recruits—representing the civilian population—and four hundred soldiers were examined in early winter and again in late spring. Their mouths were examined by the late Brigadier H. Stobie, Consulting Dental Surgeon to the Army, whose report showed no connexion between incidence of gingivitis and vitamin C reserve in these 1200 men. There was no scurvy.

Recruits showed more vitamin C than soldiers, so that even in spring their reserves were greater than those of the soldiers had been 4 months previously in winter.

Both recruits and soldiers from the country were superior to those from industrial areas. Only 1 % of recruits remained unsaturated after five or six doses of synthetic vitamin C, whereas in spring soldiers of Scottish Command (Glasgow) showed 34 % unsaturated after five doses and 21 % after six. It appeared desirable to increase the vitamin C supply to troops in winter. One test with forty-three men showed that saturation was reached more rapidly in those who had the vitamin dose after breakfast rather than before it.

W.R.G.A.

THE RELATION BETWEEN THICKNESS OF CLOUD LAYERS AND THEIR TRANSMISSION OF LIGHT

By **W. R. G. Atkins**

Quart. Journ. Roy. Met. Soc., Vol. 77, 1951, pp. 659–62

Apparent minimal cloud-transmission percentages for Bircham Newton, Norfolk, are plotted against vertical distribution of clouds observed from aircraft, and tenths of cloud cover, the latter being indicated by modifications in the line joining the points showing base and top of each cloud layer. With under 20 % transmission great cloud thickness is possible, but with over 30 % no great thickness of low cloud occurs. Percentage frequencies of minimal cloud transmission are given for a year. Making use of Hewson's calculations for reflexion, absorption and transmission of solar radiation for droplets of 40 μ (fog) and 10 μ (cloud) for various water contents it was found that the observed transmissions of morning fog at Bircham Newton in May were in accord with 1·0 g./m.^{3} water content, whereas transmission by thick cloud during a depression in May, and by layers of cloud another day were probably due to droplets of 10 μ at 0·1 g./m.^{3} concentration. W.R.G.A.

PHOTOELECTRIC MEASUREMENTS OF THE SEASONAL VARIATIONS IN DAYLIGHT AT PLYMOUTH FROM 1947 TO 1949

By **W. R. G. Atkins** and **Pamela G. Jenkins**

Quart. Journ. Roy. Met. Soc., Vol. 78, 1952, pp. 70–5

Daylight received on a horizontal surface has been measured for 14 years using a Burt vacuum sodium cell and a Cambridge thread-recorder galvanometer. Mean monthly values of daily maxima, in kilolux, are tabulated, also mean monthly values of illumination in kilolux hours. These are compared with similar measurements at Kew in 1947 and 1948, got with a selenium cell. The kilolux hours for each month are shown as a percentage of individual years, with mean values for 14 years and percentage range for variation. Annual totals were 108, 124 and 122 megalux hours for 1947, 1948 and 1949 respectively with 116 as the mean of the whole series. Between these and sunshine or rainfall there is no simple relation. Cloudless days are not the brightest owing to downward reflexion from clouds. Darkest days in each month commonly show 17–40 % as many kilolux hours as do brightest, though smoke pollution may reduce illumination to 5 %. W.R.G.A.

MUSCULAR AND HYDROSTATIC ACTION IN THE SEA-ANEMONE *METRIDIUM SENILE* (L.)

By **E. J. Batham** and **C. F. A. Pantin**

Journ. Exp. Biol., Vol. 27, 1950, pp. 264–89

The mechanics of muscular action and hydrostatic action are discussed in the sea-anemone *Metridium senile.* This animal exhibits a great variety of body shapes which are brought about by continual slow muscular activity. The action of most of the muscles is very slow, isotonic contraction of the parietal muscles requiring 40–60 sec. to reach its maximum and many minutes to relax. The body-wall is capable of extension of about 400 % but there are limits to extensibility in the normal animal. The mechanisms by which the animal itself increases or reduces extension by controlling its coelenteric volume are described. Pressure changes in the coelenteron which occur during activity show that both retraction and extension of the column are active processes involving a rise in pressure which enforces reciprocal extension of the opposing musculature. The relation of normal activity and shape to the coelenteric pressure is shown. This average pressure is extremely small, about 2–3 mm. of water. In a moderately filled unstimulated animal the natural muscular contractions are accompanied by a rise in pressure not generally exceeding 6–7 mm. of water. The isometric pressure which the body-wall can develop in the coelenteron has been estimated. Pressures developed during natural contractions demand muscular tensions in the body-wall ranging between 20 and 50 % of the isometric tension. An estimate is deduced from the coelenteric pressure of the isometric tension developed by the circular muscle of the column. This is about 3·5 g./cm. of body-wall transverse to the muscle. Extensive responses of the powerful retractor muscles involve much greater pressures than those against which the column muscles can operate. Development of these muscles is related

to the necessity of speed of action. Muscular action in a hydrostatic skeletal system is contrasted with that in the jointed skeletal system of vertebrates and arthropods.
C.F.A.P.

INHERENT ACTIVITY IN THE SEA-ANEMONE *METRIDIUM SENILE* (L.)

By **E. J. Batham** and **C. F. A. Pantin**

Journ. Exp. Biol., Vol. 27, 1950, pp. 290–301

Methods of observing and analysing continual muscular activity of the sea-anemone *Metridium senile* are discussed. This activity is so slow that it is rarely appreciated by the eye as movement. The activity of the column consists of a sequence of reciprocal contractions of the parietal muscles and the circular muscle coat. The activity of different parts of the body-wall may show striking co-ordination, a contraction of one part of the parietal musculature is usually followed by a contraction of the others. In other cases there may be no trace of co-ordination. Co-ordination takes place through one part of the body-wall acting as leader. The other parts of the body-wall follow this contraction with long delays. There is evidence that the delay is of local origin. One sector usually maintains leadership for long periods but from time to time the site of leadership changes. Evidence is given that the activity is inherent and continues unaltered in the absence of external stimulation. There is considerable variation in character and extent of activity in different animals and at different times.
C.F.A.P.

PHASES OF ACTIVITY IN THE SEA-ANEMONE *METRIDIUM SENILE* (L.), AND THEIR RELATION TO EXTERNAL STIMULI

By **E. J. Batham** and **C. F. A. Pantin**

Journ. Exp. Biol., Vol. 27, 1950, pp. 377–99

Basic activity in the sea-anemone *Metridium senile* has been studied and analysed in detail. Under constant conditions the animal exhibits continual slow inherent activity. The pattern of this activity varies in character from time to time and these different patterns of activity have been termed phases. A change of phase may be initiated by certain stimuli such as ingestion of food. In contrast with the direct responses such as the retraction reflex, the stimulus does not directly maintain a phasic response; it merely initiates a new phase, and the activity pattern of the latter is maintained long after the initiating stimulus. Phase changes also differ from simple reflex in that the threshold for their initiation varies enormously in different animals and in the same animal at different times. Locomotion is another phasic activity which may be initiated by various stimuli or may take place spontaneously in the absence of evident external stimuli. The alternating phases of expansion and contraction occur frequently and their relation to diurnal and other rhythms is discussed. Daily illumination can often initiate and control regular daily phases of contraction. In complete darkness and constant environmental conditions, alternating phases of expansion and contraction may still take place. It appears that a periodic stimulus such as daily illumination acts by 'setting the pace' of this inherent alternating phase change. Experiments show that continual and varying patterns of inherent activity play an important part in the behaviour of *Metridium*. Phasic activities are less easily observed than direct responses because they are so slow, but they play an essential part in behaviour patterns such as food capture, and they tend to be relative to a future possible event rather than to a past stimulus. C.F.A.P.

THE ORGANIZATION OF THE MUSCULAR SYSTEM OF *METRIDIUM SENILE*

By **E. J. Batham** and **C. F. A. Pantin**

Quart. Journ. Micr. Sci., Vol. 92, 1951, pp. 27–54

The muscular system of *Metridium* consists of fields of relatively short muscle fibres. These form a connected network and can shorten to about a fifth of the extended length. Deformation of the body-wall is described and is shown to be controlled in part by the contractility of the muscle-fibres, and in part by the properties of the mesogloea. A longitudinal contraction of the body-wall is accompanied by thickening of the mesogloea and buckling which throws the layer of circular muscles into folds. A natural limit to the extension of anemone tissue is reached when the muscle-layer is completely unbuckled. The function of the muscle-fields is analysed and the muscular plan of the pedal disk is compared with the tube foot of *Asterias*. A significant functional similarity is found in the operation of vertical, oblique and radial muscles bearing on the adhesive disk. Finally, the functional organization of the oral disk and tentacles is discussed. C.F.A.P.

ULTRAVIOLETTABSORPTION DER MEERESALGEN

By **Richard Biebl**

Ber. Deutsch. Bot. Ges., Bd. 65, 1952, pp. 37–41

In the summer of 1951, at the Marine Biological Laboratory in Plymouth, experiments were made with a Unicam photoelectric quartz spectrophotometer to determine the absorption of the green alga *Ulva lactuca*, the red algae *Porphyra umbilicalis* f. *laciniata*, *Phycodris rubens*, *Polyneura hilliae*, and of the brown alga *Dictyota dichotoma*, by exposing their thalli to light of wave-length ranging from 400 to 800 mμ and to ultraviolet rays of 200–400 mμ.

The resulting diagrams show that the absorption of visible light corresponds to what has already been recorded in the literature. The results of the measurements on the absorption of light in the ultraviolet region, however, are new. They show characteristic absorption curves between 290 and 400 mμ for each alga, though with partly opposite trends for red algae and green algae. But typical absorption of green, red, and brown algae could be found when exposing them to light of wave-lengths ranging from 200 to 290 mμ. Such absorption curves are flat and run almost parallel.

It is of ecological interest that these findings fit the fact that algae in their natural surroundings are at best exposed to rays of 300 mμ upwards, whereas they are never reached by shorter wave lengths. R.B.

SOME EXPERIMENTS WITH THE COMMON HERMIT CRAB (*EUPAGURUS BERNHARDUS* LINN.), AND TRANSPARENT UNIVALVE SHELLS

By L. R. Brightwell

Proc. Zool. Soc. Lond., Vol. 121, 1951, pp. 279–83

Between 10 and 24 July 1950, experiments were made at the Plymouth Laboratory with glass univalve shells, as originated by the late Richard Elmhirst at Millport. The shells used were perfect replicas of *Buccinum* and the work of Mr J. G. Brett of Rugby.

Eupagurus readily accepted the shells, often leaving a natural shell for the counterfeit. The commensal worm (*Nereis fucata*) similarly accommodated itself. It invariably approached from behind, climbing over the body whorl and slipping over the lip with utmost stealth, the hermit being normally antagonistic to it. Once safe within the apical whorls the worm is accepted with indifference, and freely helps itself to food from between the pagurid's foot-jaws. The worm can be independent of the crab indefinitely, if well supplied with food.

Tests seemed to show that, of potential enemies, swimming crabs at least rely much on sight. A ravenous *Portunus puber* at once rushed at the naked abdomen of a hermit, but was baulked by the glass shell. *Eupagurus*, when hungry, will freely search the interior of the anemone *Caliactis*, as encountered attached to the borrowed shell of another hermit, or living, as it often does, quite independently.

A hermit having the terminal joint of its great claw crushed did not discard it at the breaking plane, but picked it clean of muscular tissue L.R.B.

THE STRUCTURE AND FUNCTION OF THE BASEMENT MEMBRANE MUSCLE SYSTEM IN *AMPHIPORUS LACTIFLOREUS* (NEMERTEA)

By J. B. Cowey

Quart. Journ. Micr. Sci., Vol. 93, 1952, pp. 1–15

The body-wall of *A. lactifloreus* has the following structure from the outside inwards.

(i) A basement membrane of five to six layers immediately underlying the epithelium. Each layer consists of right-hand and left-hand geodesic fibres making a lattice, whose constituent parallelograms have a side length of from 5 to 6μ. The fibres are attached to one another where they cross; so there can be no slipping relative to one another.

(ii) A layer of circular muscle fibres running round the animal containing two systems of argyrophil fibres—one of fibres at intervals of 10μ running parallel to the muscle-fibres and the other of fibres running radially through the layer from the basement membrane to the myoseptum.

(iii) A myoseptum which is identical in structure with a single layer of the basement membrane.

(iv) A layer of longitudinal muscle, whose fibres are arranged in layers on each side of a series of longitudinal radial membranes.

Membranes identical in structure with the basement membrane invest the nerve cords, the gut, the gonads, and the proboscis.

The interrelations of argyrophil and muscle fibres in the muscle layers are described and their functioning discussed.

The system of inextensible geodesic fibres is analysed from a functional standpoint. The maximum volume enclosed by a cylindrical element (cross-section circular), of such a length that the geodesic makes one complete turn round it, varies with the value of the angle θ between the fibres and the longitudinal axis. When θ is 0° the volume is zero; it increases to a maximum when θ is 54° 44′ and decreases again to zero when θ is 90°. The length of the element under these conditions varies from zero when θ is 90° to a maximum (the length of one turn of the geodesic) when θ is 0°.

The body volume of the worm is constant. Thus it has a maximum and minimum length when its cross-section is circular and at any length between these values its cross-section becomes more or less elliptical. It is maximally elliptical when θ is 54° 44′, i.e. when the volume the system could contain, at circular cross-section, is maximal. From measurements of the ratio of major to minor axes of this maximally elliptical cross-section, the maximum and minimum lengths of the worm relative to the relaxed length and values of θ at maximum and minimum lengths are calculated. The worm is actually unable to contract till its cross-section is circular; but measurements of its cross-sectional shape at the minimum length it can attain, permit calculation of the theoretical length and value of θ for this cross-sectional shape.

Calculated values of length and the angle θ agree well with the directly observed values. J.B.C.

THE BLOOD SYSTEM IN THE SERPULIMORPHA (ANNELIDA, POLYCHAETA). I. THE ANATOMY OF THE BLOOD SYSTEM IN THE SERPULIDAE. II. THE ANATOMY OF THE BLOOD SYSTEM IN THE SABELLIDAE, AND COMPARISON OF SABELLIDAE AND SERPULIDAE. III. HISTOLOGY

By **Jean Hanson**

Quart. Journ. Micr. Sci., Vol. 91, 1950, pp. 111–29; pp. 369–78; Vol. 92, 1951, pp. 255–74

The anatomy and histology of the blood system in a number of serpulids and sabellids from Plymouth and Naples have been investigated: *Serpula*, *Hydroides*, *Vermiliopsis*, *Pomatoceros*, *Salmacina*, *Protula*, *Apomatus*, *Spirorbis*, *Sabella*, *Potamilla*, *Branchiomma*, *Dasychone*, *Amphiglena*, *Fabricia*, *Jasmineira*, *Dialychone* and *Myxicola*. In both families one can distinguish a central blood system in which a true circulation is probably maintained, and a peripheral blood system of predominantly blind-ending vessels which are alternately empty and full, receiving their blood from the central system and then returning it along the same channels. The central blood system is built on the same plan in both families, but the peripheral system is variable, especially amongst the sabellids. The variations are partly attributable to differences in body size. The functions of subepidermal and coelomic capillaries and the blood supply to the muscles of the body-wall are discussed. In both families all vessels possess a three-layered wall consisting of an endothelium, a skeletal coat, and a peritoneum containing muscle fibres which lie transversely to the long axis of the vessel. The structure of these three coats is described in detail. Blood cells are absent in all genera. J.H.

THE SODIUM AND POTASSIUM CONTENT OF CEPHALOPOD NERVE FIBRES

By **R. D. Keynes** and **P. R. Lewis**

Journ. Physiol., Vol. 114, 1951, pp. 151–82

A method is described for determining sodium and potassium simultaneously, in quantities down to 0·3 and 3 μg. respectively. The technique used was 'activation analysis', which involved irradiation of the tissue samples in a neutron pile for a week, followed by β- and γ-counts to determine the amounts of ^{42}K and ^{24}Na which had been formed.

Figures were obtained for the sodium and potassium concentrations in freshly dissected 200 μ *Sepia* axons, and for the rate of loss of potassium and gain of sodium in axons kept in sea water at about 10° C. Estimates for a further series of axons which had conducted about 120,000 impulses before being analysed, showed that during activity there was a net gain of 3·8 $\mu\mu$mol. Na/cm.^{2}/impulse, and a net loss of a roughly equal quantity of potassium. Analyses of samples of axoplasm extruded from 500 μ squid axons gave very similar results. Measurements of the chloride content of the axoplasm samples showed a considerably smaller net gain on stimulation.

These results provide direct confirmation for the 'sodium hypothesis' of nervous conduction, put forward by Hodgkin, Huxley & Katz. R.D.K.

Colours of Marine Animals

By **J. A. Colin Nicol**

School Science Review, Vol. XXXIII, 1952, pp. 208–18

A general account is given of coloration in marine animals, and of the pigments and structural modifications which are responsible. Either may be responsible for the colour of a particular animal, or both may play a complementary role. The principal pigments responsible for external colours are carotenoids, porphyrins, indole pigments, melanins, quinones, purines, and various protein substances. The distribution and biological implications of some of these substances are discussed. Structural colours are due to interference effects, and may be seen in the iridescence of various animals. Morphological and physiological colour changes are distinguished, and the significance of adaptive coloration to certain species is considered. The article concludes with a series of references which provide an introduction to the bibliography dealing with the subject. J.A.C.N.

A New Subterranean Gammarid (Crustacea) from Britain

By **G. M. Spooner**

Proc. Zool. Soc. Lond., Vol. 121, 1952, pp. 851–9

Niphargus glenniei is a new species of the subterranean genus *Niphargus* discovered in limestone caves near Buckfastleigh, S. Devon, where it was first collected by Brig. E. A. Glennie of the Cave Research Group. A detailed description is given. The species is small, and the male sex has not yet been recognized.

The nearest possible relatives on the continent are *N. arndti*, known from four localities in Silesia, and *N. nolli*, known from two localities in the upper Rhineland, both small species in which the mandibular palp shows an unusual simplification. However, differences between *N. glenniei* and these two species are considerable, and the resemblances may conceivably be but the effect of convergence.

Schellenberg's genus *Niphargellus* is not to be considered valid, an opinion enhanced by the discovery of *Niphargus glenniei*.

The existence of this distinct new species was indeed unexpected, in view of the poverty of the British subterranean arthropod fauna and the lack of endemic forms.
G.M.S.

THE RESPIRATORY SIGNIFICANCE OF THE CROWN IN THE POLYCHAETE WORMS *SABELLA* AND *MYXICOLA*

By G. P. Wells

Proc. Roy. Soc., B., Vol. 140, 1952, pp. 70–82

The tube of *Sabella pavonina* is irrigated by means of piston-like waves travelling tailwards along the worm's body, a process which is hardly affected by decapitation. The tube of *Myxicola infundibulum* is not irrigated at all. After bisection of tubeless worms at the junction of thorax and abdomen, the oxygen consumption of both halves together is greatly depressed in *Myxicola* but is unaffected in *Sabella*. The conclusion is drawn that the body of *Myxicola* depends largely on the crown for its oxygen supply while that of *Sabella* normally derives its oxygen from the irrigation current through the tube. The results are discussed in relation to the differences in anatomy, and in the ability to survive and regenerate after injury, between the two worms.
G.P.W.

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